Population biology of rare plants: the effects of ecological and genetic processes for the growth and viability of populations of three endangered floodplain violets

Populationsbiologie seltener Pflanzenarten: Effekte ökologischer und genetischer Prozesse für das Wachstum und die Vitalität von Populationen dreier gefährdeter Stromtalveilchen

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"A biology of populations is a study of the numbers of organisms. (...) It is concerned with the life cycles of organisms because a population cannot be adequately described without taking into account that it may include young and old, big and small, male and female."

(John L. Harper 1977)

Contents

Chapter 1

Introduction

Since the evolution of mankind, humans have – as most other organisms – modified their environment. Large parts of the world's land surface have been cultivated and subject to human land use for at least some thousands of years (e.g., Jankuhn 1969). With the development of agriculture and the first farming systems, new species of crops and weeds developed or were transported across their natural boundaries. Through the cultivation of land by means of melioration of environmental conditions and the clearing of the dominant forests new habitats were created or their area enlarged (e.g., Firbas 1949; Ellenberg 1986). Especially within the emerging cultural landscape of Europe this development led to a considerable increase in the diversity of environmental conditions, habitats and, consequently, plant and animal species.

However, during the last decades human domination of ecosystems and global processes have reversed this development and we currently witness ever increasing rates of species extinction (e.g., Vitousek *et al.* 1997; Sala *et al.* 2000) with negative effects on global and regional biodiversity and ecosystem functions (Chapin *et al.* 1997). Worldwide, about 25% of the vascular plant species may become extinct within the next decades (Raven 1987, as cited in Schemske *et al.* 1994), and in Germany 28.4% of the total flora of flowering plants and ferns of 3319 species is red-listed (Korneck *et al.* 1996); since 1850 about 47 species have gone extinct.

Globally, human land-use changes are expected to exert the largest effects on biodiversity (Chapin *et al.* 1997; Vitousek *et al.* 1997; Sala *et al.* 2000). While land-use changes on this scale denote the total conversion of ecosystems, e.g. from tropical forest to grassland or from grassland to crop fields, there may also be profound changes in ecosystem structure, function and composition as a consequence of intensification or de-intensification of land-use, especially within the cultural landscape of Europe. Here, intensification of land-use will lead to direct negative effects through increased disturbance, fertilisation and the application of biocides and lead to a homogenisation of landscapes. Abandonment of land-use and the following unguided succession processes will displace species of semi-natural habitats adapted to regular management. As a consequence of intensification and abandonment that led to increased fragmentation of

habitats and the isolation of populations, many (also formally widespread) species have become rare and restricted to small and/or isolated populations (e.g., Saunders *et al.* 1991; Honnay *et al.* 2005).

Rarity, range and abundance in plants

There is large variation in geographic range and local abundance among plant species (Hanski *et al.* 1993; Lawton 1993; Thompson *et al.* 1998). Generally, we find a positive correlation between range size and population abundance, i.e. species with large geographic ranges tend to be locally abundant, while species with restricted geographic range and/or narrow habitat specificity are classified as 'rare' (Rabinowitz 1981; Hanski *et al.* 1993; Lawton 1993; Gaston 1997; Thompson *et al.* 1998). Rarity, in turn, is closely related to the species risk of extinction (Fischer & Stöcklin 1997; Gaston 1997; Matthies *et al.* 2004) because species with narrow niche breadth, few or small populations are most likely to suffer from variation or directional changes in external (e.g., climate, habitat fragmentation, management, introduced species) or internal conditions (e.g., random mortality, success of pollination, pollen source, reproduction) than widespread and common, generalist species (Gaston & Kunin 1997a). Rarity is taxonomically biased (Schwartz 1993) and positively related to taxon size (Schwartz & Simberloff 2001). Despite their subordinate position in rank-abundance plots, the loss of rare species (and the decrease in diversity) may considerably impair ecosystem functions (e.g., Lyons & Schwartz 2001; Lyons *et al.* 2005).

There are some generalities that emerged from the accumulating body of literature on rare-common differences during the last decades.

Very broadly, rare species tend to

- be selfing or rely on vegetative reproduction (Kunin & Gaston 1993; Gaston & Kunin 1997a),
- have lower reproductive allocation (Kunin & Gaston 1993; Gaston & Kunin 1997a),
- have poorer dispersal abilities (Kunin & Gaston 1993; Gaston & Kunin 1997a),
- have lower levels of heterozygosity (cf. Gitzendanner & Soltis 2000),
- use less common resources and/or a narrower range of resources (Kunin & Gaston 1993; Gaston & Kunin 1997a),
- be smaller (at least non-woody species; Hedge & Ellstrand 1999), and
- show higher dormancy levels, higher temperature requirements for germination and asynchronous germination (Hölzel & Otte 2004a).

However, since differences in size, competitive ability (Moora *et al.* 2003), genetic variation (Gitzendanner & Soltis 2000) and the other above mentioned traits are often relatively small and studies on rare-common differences are tainted with statistical and methodological problems (e.g., Kunin & Gaston 1993; Gaston & Kunin 1997a, b; Bevill & Louda 1999) their predictive power is low and there is no "discrete set of rare species characterized by a suite of consistent traits" (Gaston & Kunin 1997b).

One of the major achievements of plant biology was the realisation that a population can not be adequately described when ignoring that it consists of individuals of different ages and stages (Harper & White 1974; Harper 1977). Since the future fate of organisms varies with age or stage, the demographic approach to conservation – population biological analyses of various aspects of the life-cycle as the fundamental unit for the description of organisms (Caswell 2001) – promises to supply the necessary information for the conservation of rare and endangered species (e.g., Boyce 1992; Menges 2000; Schemske *et al.* 1994; Caswell 2001; Morris & Doak 2002).

Which processes affect population viability?

The growth and viability of populations ultimately depend on the vital rates, i.e. *survival*, *growth* and *reproduction* (Caswell 2001). The rates of birth, growth and death are therefore central in plant population and conservation biology (e.g., Harper 1977; Silvertown & Lovett Doust 1993; Caswell 2001). These vital rates can be affected by both deterministic and stochastic processes (Box 1).

Deterministic processes such as overexploitation, land-use changes, habitat destruction and habitat fragmentation, which may cause population extinction within relatively short time, have been identified as main drivers of global biodiversity (Sala *et al.* 2000). However, also random (*stochastic*) processes, for which future states can only be described by a probability distribution, may have a strong influence on vital rates and population viability (Fig. 1.1).

Box 1. Types of stochasticity

Demographic stochasticity
Variability in population growth rates resulting from random differences among individuals in survival and reproduction within a season.

Environmental stochasticity
Random variation in birth and death rates between years in response to climatic conditions, competition, predation, pathogens or other external factors. **Catastrophes** are an extreme form of environmental stochasticity.

Genetic stochasticity
Variation in the genetic composition of a population owing to random factors (i.e. genetic drift). Genetic stochasticity is not related to systematic forces (selection, inbreeding, or migration).

Environmental stochasticity, i.e. random perturbations of external factors (biotic and abiotic), affects the vital rates of all individuals within the same age or stage class. Demographic stochasticity, i.e. random differences between individuals in the realisation of probabilities of reproduction and death, directly influence birth, growth and death rates of the population. However, since individual events level off in large populations, demographic stochasticity is most important in small populations (Lande 1993). Genetic stochasticity, i.e. genetic drift, is linked to genetic diversity and will affect population viability through its effects on the evolutionary potential of a species.

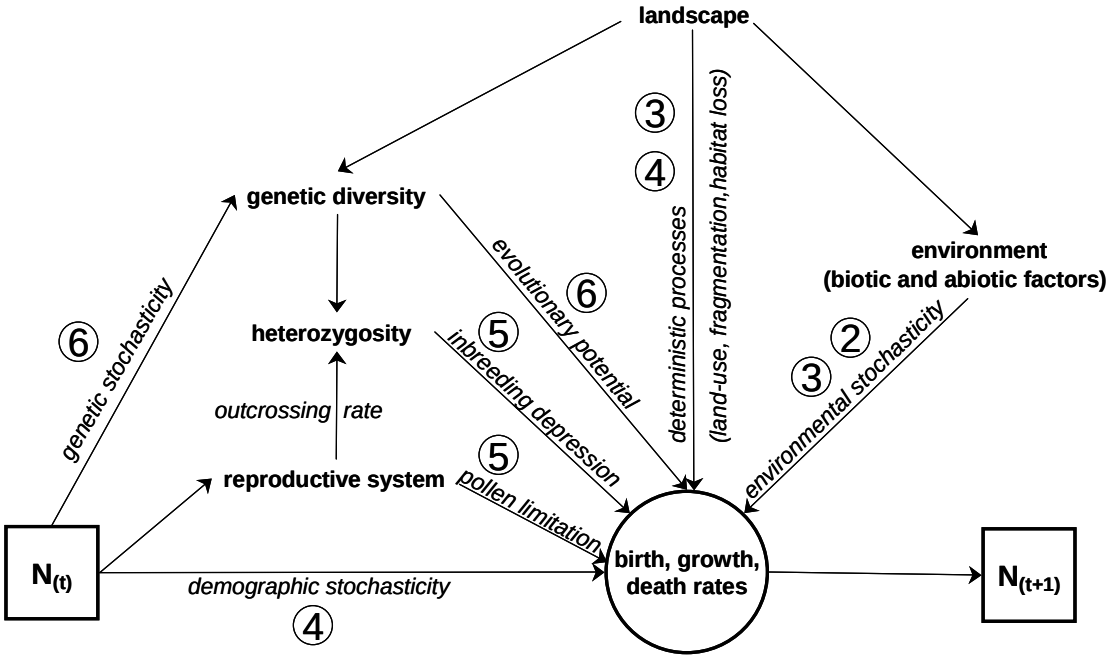

Figure 1.1 Schematic presentation of ecological and genetic **factors** and *processes* influencing the dynamics of local plant populations. Population sizes at times *t* and *t+1* are represented by boxes. Numbers denote Chapters of the present work dealing with the respective processes. According to Schemske *et al.* (1994), modified.

Although the role of genetic factors for population extinction has been controversial (e.g., Lande 1988), there is empirical evidence that inbreeding depression and loss of genetic diversity may contribute to the extinction risk in plants and animals (Frankham 2005).

The approach to use empirical data on the whole life-cycle and modelling scenarios to analyse possible effects of random and deterministic factors in order to assess the viability and future persistence of populations has been designated *population viability analysis* (PVA; e.g., Boyle 1992). Owing to the development of species management plans

based on the 'Endangered Species Act', PVAs have been very influential in conservation in North America (Menges 1990; 2000; Menges & Dolan 1998). During the last years they have been increasingly used in species conservation because they provide detailed information on the ecology and population biology of both rare and introduced species that can be used for management and control measures (e.g., Oostermeijer 1996; Pfab & Witkowski 2000; Lennartsson & Oostermeijer 2001; Morris & Doak 2002; Honnay *et al.* 2005; Münzbergová *et al.* 2005; Nicolè *et al.* 2005).

Central versus marginal populations

Within species the proportion of occupied sites and average population densities decline from the centre to the margin of its range (e.g., Lawton 1993; but see Sagarin & Gaines 2002 for a critical review). Range margin populations can be *geographically* marginal (peripheral) or *ecologically* marginal (Lesica & Allendorf 1995); in many cases the ecological conditions in peripheral populations will be different from those in central populations. Although the study of species ranges and the analysis of causes for distribution limits have traditionally been a topic of plant geography, patterns and ecological processes at the range margin have also received attention from plant ecologists (e.g., Carter & Prince 1981; Bengtsson 1993; Nantel & Gagnon 1999; Kluth & Bruelheide 2005), conservationists and plant geneticists (Safriel *et al.* 1994; Lesica & Allendorf 1995 and references therein; Durka 1999; Lammi *et al.* 1999; Lönn & Prentice 2002; Van Rossum *et al.* 2003).

Low habitat quality at the range margin may affect species performance and reduce reproduction and dispersal (Pigott & Huntley 1981; García *et al.* 2000). Additionally, the habitat type or safe-sites for germination may be infrequent at the range margin (Dinsdale *et al.* 2000; Jump & Woodward 2003). Therefore, peripheral plant populations will often be

- more isolated (Lawton 1993; Lesica & Allendorf 1995) and
- contain less individuals than core populations (Durka 1999; Lammi *et al.* 1999; but see Kluth & Bruelheide 2005).

The viability of these populations may also be reduced because of the increased chance of mating between relatives (Menges 1991; Fischer & Matthies 1998). Since pollen may originate from anthers of the same flower (autogamous selfing), from a different flower on the same shoot or genet (geitonogamous selfing), or a shoot from another genet (xenogamy, crossing) (Richards 1997), especially in small and/or isolated populations

there is a large probability for self pollination (Barrett & Kohn 1991; Dudash & Fenster 2000). Selfing may lead to inbreeding depression (e.g., Husband & Schemske 1996; Culley 2000; Dudash & Fenster 2000; Charlesworth 2003), i.e. the reduction in viability and fitness (Waller 1984; Mitchell-Olds & Waller 1985; Dudash 1990; Carr *et al.* 2003) in selfed as compared to outcrossed progeny. This may lead to increased abortion of ovules, and reduction in seed set, germination, offspring fitness and negative effects on population genetic structure (Waller 1984; Dudash 1990; Fischer & Matthies 1997; Richards 1997; Berg & Redbo-Torstensson 1999; Culley 2000; Karrenberg & Jensen 2000; Lienert & Fischer 2002).

Additionally, small and/or isolated populations may suffer from pollinator limitation (e.g., Jennertsen 1988; Ågren 1996; Fischer & Matthies 1997; Karrenberg & Jensen 2000), which may further reduce seed production (Morgan 1999). Therefore, both genetic diversity and the mating system of the species are closely linked to the degree of heterozygosity of the population (Fig. 1.1).

Aims of the present work

The present work contains scientific studies on different aspects of the population biology of three rare plant species of floodplain meadows. The congeneric *Viola elatior* Fries, *V. pumila* Chaix and *V. stagnina* Kit. are endangered at a European scale and red listed in many countries (Korneck *et al.* 1996; Schnittler & Günther 1999). Based on the size and position of their total range, the responsibility of Germany for the conservation of these species is moderate (*V. elatior*, *V. pumila*) to very large (*V. stagnina*) (Floraweb, BfN 2005). Additionally, the communities where theses species occur, i.e. low-input hay meadows of the Cnidion and Molinion type, are among the most endangered plant communities in Germany (Floraweb, BfN 2005).

The main aim of this work was to apply an experimental demographic approach to the conservation of these three species. Specifically, I intended to

- analyse the influence of (cf. Fig. 1.1)
  - ❖ demographic, environmental, and genetic stochasticity, and
  - ❖ deterministic processes (management, fragmentation) on population growth and viability,
- describe and compare the species life-cycle in central and marginal populations,
- analyse the genetic population structure and the relative importance of genetic drift and gene flow in central and marginal populations,

- analyse the effects of mating system and pollen source on seed production and offspring performance, and
- identify sensitive stages in the life-cycle.

Overview of the chapters

Chapter 2 describes a field experiment with planted seedlings of the three species that analyses the joint influence of diffuse competition through the surrounding meadow vegetation and water availability, i.e. *environmental stochasticity* (Fig. 1.1), on the species vital rates (survival, growth and reproduction). **Chapter 3** will focus on the effects of management (*deterministic process*) and environmental variation (*environmental stochasticity*) on the population stage structure of the species. In **Chapter 4** the life-cycle of the three species in central and marginal populations is compared. Differences between the study species, the study regions, and the effects of *demographic stochasticity* are analysed and their implications for population viability and conservation discussed. The study species are true cleistogamous plants (Plitmann 1995), i.e. an individual may bear both open, potentially cross-pollinated and closed, obligatorily selfed, cleistogamous capsules. Therefore, **Chapter 5** scrutinises possible effects of the cleistogamous mating system on seed production and seedling performance. It thus addresses the question of pollen limitation and inbreeding depression (Fig. 1.1). **Chapter 6** will focus on *genetic stochasticity* and the relative roles of genetic drift and gene flow for population genetic structure. This chapter will test predictions by the theory of range margins (Soulé 1972; Lawton 1993; Sagarin & Gaines 2002) with respect to population size, isolation and genetic divergence. **Chapter 7** contains an extended summary of the results presented in **Chapters 2-6** and the main conclusions of the work with respect to the general and specific aims listed above. **Chapter 8** presents the extended summary translated to German. *References* to literature used throughout this work (**Chapters 1-8**) are collected in a common reference list at the end of this work.

Chapter 2

Differential effects of interspecific interactions and water availability on survival, growth and fecundity of three congeneric grassland herbs

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Abstract

Fitness of individual plants and of populations depends on the rates of survival, growth and fecundity. In this paper I tested whether vital rates were differentially affected by biotic interactions and water availability.

The effects of manipulations of above-ground competition (through clipping) and water-availability (through water-addition) on the vital rates of seedlings of three species were analysed in dry, mesic and wet grassland.

Water-addition and grassland type had the largest effects on survival (accounting for 41 and 24% of total variation, respectively) across species. Height growth rate was positively affected by grassland type (19%), water-addition (12%) and varied among species (8%), while leaf accumulation rates and reproduction were affected by grassland type and clipping. The data suggested facilitative effects of the canopy on seedling survival in the dry grassland.

This study presents evidence that environmental conditions and biotic interactions may have differential effects on seedling survival, growth and reproduction. The findings highlight the complex interplay between spatial and temporal environmental variation and biotic interactions in structuring plant communities.

Introduction

Fitness of individuals and populations will depend on the rates and probabilities of (i) *survival*, (ii) *growth* and (iii) *fecundity* under different environmental conditions ('vital rates' *sensu* Caswell 2001). Only those individuals that survive will be able to grow and there is often a threshold size that has to be reached before reproduction occurs (e.g., Mendéz & Obeso 1993; Schmid *et al.* 1995; Mendéz & Karlsson 2004). As a consequence of stressful environmental conditions mortality increases and biomass production decreases especially in small individuals (e.g., De Jong & Klinkhamer 1988; Carlsson & Callaghan 1994; Rousset & Lepart 2000). On the other hand, leaf traits and reproduction appears to be especially responsive to experimental manipulations of light interception and neighbour removal (e.g., Mattila & Salonen 1995; Graglia *et al.* 1997; Jensen & Meyer 2001; Rousset & Lepart 2000).

Interspecific interactions among plants are recognised as an important determinant of community structure, composition and dynamics (Grace & Tilman 1990; Bertness & Callaway 1994; Callaway 1995; Grime 2001; Keddy 2001), though there has been much debate about the processes of competition and traits of successful species (Bengtsson *et al.* 1994; Aerts 1999; Grime 2001; Keddy 2001). While earlier studies emphasised the prevalence of negative interspecific interactions (competition) for structuring plant communities (e.g., Connell 1983; Schoener 1983), more recently evidence for the important role of positive interactions (facilitation) among plants is accumulating (Bengtsson *et al.* 1994; Bertness & Callaway 1994; Callaway 1995; Bertness 1998). While facilitation was first found in habitats characterised by extreme environmental conditions (dunes: De Jong & Klinkhamer 1988; alpine tundra: Carlsson & Callaghan 1991; salt marsh: Bertness & Hacker 1994; montane or dry calcareous grasslands: Ryser 1993; Greenlee & Callaway 1996; Rousset & Lepart 2000; desert: Tielbörger & Kadmon 2000), new synthetic approaches emphasise that there is a balance between positive and negative interactions (Callaway & Walker 1997; Brooker & Callaghan 1998; Dormann & Brooker 2002) and, consequently, that these processes may occur simultaneously and only their net effect will vary along environmental gradients (Holmgren *et al.* 1997) or between years (Greenlee & Callaway 1996; Tielbörger & Kadmon 2000).

The incorporation of negative *and* positive plant interactions has been proposed to change paradigms in plant community ecology (Lortie *et al.* 2004). Local environmental conditions and plant interactions represent two of the main filters that determine

diversity and composition of extant plant communities (Grime 1998; Lortie *et al.* 2004). Their interplay that varies in time and space may be a key for understanding species coexistence in plant communities (e.g., Silvertown *et al.* 1999). However, to my knowledge there are only few examples where the importance of environmental conditions and interspecific interactions (positive and negative) for survival, growth and reproduction of plant species have been evaluated separately (but see De Jong & Klinkhamer 1988; Hastwell & Facelli 2003).

The aim of the present paper was to test whether diffuse above-ground competition and water availability differentially affected survival, growth and reproduction of seedlings of three congeneric herbs along a water availability gradient in mesic temperate grassland. I manipulated above-ground competition through repeated removal of vegetation surrounding planted seedlings and ameliorated water stress by regular water-addition.

The hypothesis was that:

(1) environmental conditions (soil water availability, water-addition) should have the largest effect on seedling survival and height growth rate, while reduced light interception as a result of interspecific interactions should have stronger effects on leaf growth and reproduction and
(2) the net effect of interspecific interactions on seedlings should vary along the soil water availability gradient, supposedly from positive in the dry grassland to negative in the wet grassland.

Material and Methods

Study species

The study species (*Viola elatior* Fries, *V. pumila* Chaix, *V. stagnina* Kit. [syn. *V. persicifolia* Schreber]) are perennial iteroparous hemicryptophytes (Quinger 1993) with a long-term persistent seed bank (Hölzel & Otte 2004b). In Central Europe the species mainly occur in the corridors of large lowland rivers. *Viola elatior* and *V. pumila* grow on base-rich soils with relatively low but seasonably variable water availability, while *V. stagnina* is found under more permanently wet, acidic conditions (Hölzel 2003). *Viola pumila* and *V. stagnina* are true meadow species of species-rich, usually regularly mown sub-continental meadows of the phytosociological units *Molinion* (low productive) and *Cnidion* (productive) (Hölzel 2003), while habitats of *V. elatior* are characterised by low intensities of disturbance. The latter occurs along of woodland fringes (class

Artemisietea) and the edges of low-productive floodplain meadows (alliance *Molinion*) (Hölzel 2003) and on wooded meadows (Moora *et al.* 2004).

Experimental design

For the present study I used cleistogamous seeds, i.e. seeds originating from closed flowers that are obligatorily selfing, of the three species from a pollination experiment (Eckstein & Otte, unpubl.). In early spring 2003, seeds were sown on commercial potting soil in trays that were kept under identical outdoor conditions in a common garden close to Giessen, Germany (50°32' N, 8°41' E, 172 m a.s.l.). In mid-May, 60 healthy looking seedlings of each species were randomly selected. Across species these were between 1 and 9 cm tall (minimum and maximum) and had between 1 and 5 true leaves. I established 60 experimental plots of about 0.1 m2 (31.5 × 31.5 cm) in each of three grassland types within about 50 m distance in the experimental garden of the Department of Landscape Ecology and Landscape Planning, Giessen University. Plots were placed in a 12 rows × 5 columns grid and protected from slugs by a special fence. A distance of 0.5 m between rows and of 0.2 m between columns assured that effects of the canopy of unclipped plots on seedlings on adjacent clipped plots was minimised. One seedling together with the soil that adhered to its root system was carefully planted into a hole (3 cm deep, 3 cm diameter) in the centre of each plot and watered. Seedlings were randomly allocated to plots. Until the start of the experiment on May 19, reserve plants replaced dying seedlings. Grasslands were selected with respect to species composition, position to the ground water table and substrate that they could be positioned along a water availability gradient representing high (wet grassland), low (dry grassland) and intermediate (mesic grassland) water availability (Table 2.1). Measurements of soil water content within the uppermost 5 cm confirmed the ranking of sites. Water contents (2-10% of soil d.wt.; Table 2.1) during a prolonged drought period were within the range of summer soil humidity in Dutch sand-dune ecosystems (De Jong & Klinkhamer 1988). In half of the plots (n=90) the above-ground vegetation was clipped at weekly intervals throughout the experiment (C+) to remove the effects of diffuse competition for light of the vegetation canopy, the other half remained unclipped (C–). Each of these groups was again randomly divided into two groups (n=45), one group receiving extra watering of 1 L every second day (W+) (an amount corresponding to 10 mm of precipitation), and the other half remained untreated (W–). The experiment thus consisted of a factorial

Table 2.1 Substrate, water content (%), number of species, characteristic species (absolute frequency in brackets), average canopy height (cm), biomass (g m^{-2})at the end of the growing season (early September) and soil pH (in water) of the three grassland types.

		Characteristic					
Grassland type	Substrate	Water content	Number of species	Species frequency	Canopy height	Biomass	pH
Dry	Sand	1.8 ± 0.3 **a**	5.6 ± 0.2 **a**	*Dianthus deltoides* (24) *Rumex acetosella* (18) *Achillea millefolium* (17)	20.9 ± 1.8 **ab**	401.2 ± 26.9 **b**	6.3 ± 0.1 **b**
Mesic	Sandy Clay	4.2 ± 0.3 **b**	4.7 ± 0.3 **b**	*Festuca rubra* (30) *Stellaria graminea* (13) *Galium verum* (7)	15.0 ± 0.9 **a**	247.5 ± 10.0 **a**	5.5 ± 0.1 **a**
Wet	Clay	9.3 ± 0.7 **c**	5.6 ± 0.2 **a**	*Agrostis stolonifera* (28) *Vicia hirsuta* (24) *Poa trivialis* (19)	22.9 ± 1.6 **b**	478.6 ± 21.1 **c**	6.3 ± 0.1 **b**
N		9	30	30	30	30	12

Data are means ± s.e., *n*=number of samples or plots analysed. Water content was measured as percentage of soil d. wt. in soil samples of 3 cm diameter and a depth of 5 cm during a prolonged period of drought. Different letters within a column denote significant differences ($p<0.05$) between grassland types (Tukey HSD test after significant one-way analysis of variance). Data were Log_e-transformed before analysis to meet assumptions of analysis of variance, if necessary. The table contains back-transformed values.

combination of three grassland types × three species × two clipping treatments × two watering treatments with five replicates per treatment combination.

Measurements and Calculations

I recorded the fate (alive, dead), the height and number of leaves and the number of seed capsules of each plant at weekly to fortnightly intervals from May 19 to September 23. These data served to separately analyse the effects of the experimental factors and their interactions on survival, growth and reproduction. Fate was used to carry out a survival analysis (Fox 1993) for the effects of experimental factors on cumulative seedling survival. The fact that the experimental sites were protected against slugs and almost all dead withered individuals could be re-covered, indicated that their death was caused by environmental stress and not by herbivory.

For the analysis of effects on growth I used the relative height growth rate (HGR, % week^{-1}), which was calculated as

$$HGR = \frac{\ln(h2) - \ln(h1)}{t2 - t1},$$

where *h1* and *h2* are the heights (cm) at the start of the experiment and at the time the seedling was last recorded alive, and *t2-t1* is the number of weeks the seedling was alive. Additionally, by replacing height by leaf number the relative leaf number growth rate (LGR, % week^{-1}) was estimated. Since the plants started as seedlings with a few leaves and grew as single shoots throughout the experiment I consider an increase in height to represent biomass accumulation (cf. Hastwell & Facelli 2003) and an increase in leaf number to be proportional to the net accumulation of leaf area of a seedling during its lifetime. Furthermore, significant differences in HGR and LGR during 2003 among (i) plants not surviving until 2004, (ii) vegetative and (iii) reproductive plants 2004 ($F_{2,177}$=29.8, and $F_{2,177}$=65.5, respectively, $p<0.0001$ in both cases; data not shown), demonstrated that these measured traits were also closely linked to future fitness.

Height is a good predictor of competitive ability (Keddy & Shipley 1989; Weiher *et al.* 1999; Keddy *et al.* 2000) and HGR will represent the ability of seedlings to escape from competition for light (cf. Lepš 1999). LGR was chosen since it represents the accumulation of photosynthetic tissue and the ability of seedlings to occupy aerial volume. I used relative rates instead of absolute values to account for (i) species-specific differences in morphology and (ii) differences in initial height and leaf number between individuals. The number of seed capsules was used as a proxy for reproductive output.

To analyse the relative strength of positive and negative effects along the environmental gradient, I calculated the relative neighbour effect (RNE) as suggested by Callaway *et al.* (2002) as

$$RNE = \frac{(Xt - Xc)}{x} * (-1),$$

where *X* is an estimate of plant performance in the absence (t) and presence (c) of neighbours, and *x* is the highest value of (*Xt*; *Xc*). This quotient is multiplied by (-1) to result in positive and negative values of RNE indicating facilitation and competition,

respectively, as suggested by Callaway *et al.* (2002). RNE ranges from –1 to +1. It was calculated for relative survival, relative height growth and relative leaf accumulation (i.e. height (leaf number) when the seedling was last recorded as alive / height (leaf number) at the start of the experiment). I only used non-watered plots for RNE, since water-addition largely removed the environmental gradient with respect to water availability. Using Monte-Carlo methods average RNE and 95% confidence intervals were estimated by bootstrapping the original data (bootstrap sample size: 10000). The obtained bootstrap distribution was then used to test whether the mean RNE differed significantly from zero (Manly 2001).

To quantify functional effects of the experimental conditions I measured photosynthetic active radiation (400-700 nm) at the top of each seedling in relation to radiation above the grassland canopy (%) on four occasions during the experiment (May 30, June 13, June 30, July 14) using a LI-190 quantum sensor (Licor Inc., USA). Furthermore, I installed two temperature-logger (Tinytalk, Gemini Data Loggers Ltd., UK) per treatment combination across species that recorded hourly temperatures about 0.5 cm below soil surface from May 20 until July 24, i.e. during the main period of seedling establishment and mortality (see below).

Canopy height (cm) of the surrounding vegetation was recorded on several occasions to document vegetation structure. Additionally, at the end of the growing season in October, the above-ground vegetation of all unclipped plots (n=30 per grassland type) was harvested, dried to constant mass at 70°C and weighed to the nearest milligram.

Data on daily precipitation (mm) during 2003 were obtained from the meteorological station of the Department of Plant Ecology about 500 m from the experimental site.

Statistics

Since survival data are censored (i.e. truncated) data, non-parametric tests are usually applied in failure-time approaches (Quinn & Keough 2002). Using the censored data on individual survival at each of the ten occasions I carried out a survival analysis as described by Fox (1993). In this method, a score is first assigned to each survival time using the procedure of Mantel (1967). Using sums (for each group) of this score, in a next step differences in cumulative survival between two (test statistic: Z) or more levels (test statistic: Chi^2) of the main factors can be tested using the non-parametric Gehan's generalized Wilcoxon test (Gehan 1965a, b).

Since differences in survival among the model species were not significant (see results), I also tested the main effects of grassland type (dry, mesic, wet), clipping (C+, C–), water-addition (W+, W–) and their interactions on seedling survival at the end of the experiment across species (n=15 per treatment combination), using a three-way general linear model analysis of variance (Quinn & Keough 2002).

The effects of species (*V. elatior*, *V. pumila*, *V. stagnina*), grassland type, clipping and water-addition on HGR, LGR and number of capsules per individual were tested in a four-way fixed effect ANOVA (Quinn & Keough 2002).

The levels of the factor grassland type clearly represented a water availability gradient. Evidence for this assumption comes from the fact that water-addition largely removed differences among grassland types (see results), and that therefore water availability was the overriding environmental factor limiting plant survival and productivity during the study year (see also De Jong & Klinkhamer 1988). Therefore, I analysed grassland type as a fixed factor (see Underwood 1997, for a discussion of random and fixed factors).

The relative importance of single factors and factor combinations in analysis of variance in relation to the total variation of the model was estimated through the 'magnitude of effects' (ω^2) (Graham & Edwards 2001). This is a measure of fit in analysis of variance, analogous to R^2 in regression analysis, which provides additional information on the importance of factors above the significance level (*P*-value).

To obtain ω^2, the variance component of each model term was calculated from the expected mean square using equations in Underwood (1997) and Graham & Edwards (2001). Magnitude of effect may also help to evaluate the importance of factors that are non additive, i.e. when there are significant interactions, since expected mean squares are particular to each model term (Graham & Edwards 2001; but see Underwood & Petraitis 1993).

For example, low relative importance of a significant interaction term may indicate that, although not statistically additive, the main effects are of greater relative importance for the response variable (Graham & Edwards 2001).

Negative variance components were replaced by zero (Graham & Edwards 2001). All statistical analyses were carried out using the program Statistica (ver. 6.0, StatSoft Inc., USA).

Results

Survival

Of 180 plants, 135 (75.0%) survived from May 19 until end of the experiment on September 23 (127 days). Seedling mortality occurred until the end of July (70 days after the start of the experiment; Fig. 2.1). Thereafter, i.e. in August and September, no further mortality was recorded and all seedlings alive by July 28 also survived until the end of the experiment. In ten cases, individuals were recorded as dead at one observation because all above ground parts had withered, but reappeared some weeks later with one or two new leaves. These plants were unambiguously identified as the result of resprouting since cotyledons were missing and were therefore coded as alive also for the previous dates. This occurred in the latter half of the growing season (late summer) when average temperatures were lower and air humidity higher than during mid-summer. Since I worked with seedlings, i.e. plants that were not yet able to accumulate resources, for the remainder of individuals that did not reappear until the end of the experiment in autumn, survival below ground appears very unlikely and these were considered dead. Their classification was confirmed by observations during the next spring when none of the plants recorded as 'dead' had re-appeared.

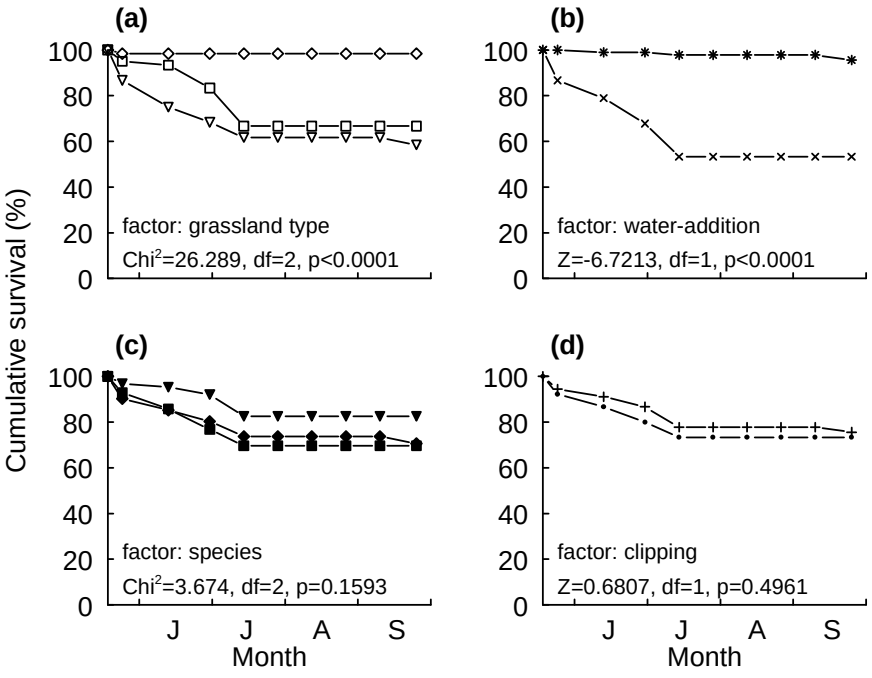

Figure 2.1 Cumulative survival (%) of individuals in dry (open triangles), mesic (open squares), and wet (open diamonds) grassland (a), in plots receiving no additional watering (crosses) and watered plots (stars) (b), belonging to different species (*Viola elatior* (filled diamonds), *V. pumila* (filled triangles), *V. stagnina* (filled squares)) (c) and from clipped (dots) and unclipped (+) plots (d). Differences between survival curves were tested using Gehans generalized Wilcoxon test (test between two groups, test statistic: Z) or an extension of Gehans generalized Wilcoxon test (test between three groups, test statistic: Chi^2). Number of individuals per group (*n*): 60 for grassland type and species, and 90 for water-addition and clipping.

Table 2.2 Effects of grassland type (G), clipping (C) and water-addition (W) on the final proportion of surviving seedlings.

Source of variation	d.f.	MS	*P*	ω^2
Intercept	1	**49.49**	**<0.0001**	
Grassland (G)	2	**1.13**	**<0.0001**	23.8
Clipping (C)	1	0.05	0.2994	0.1
Water-addition (W)	1	**3.76**	**<0.0001**	40.7
G*C	2	0.16	0.0417	2.5
G*W	2	**0.68**	**0.0005**	13.8
C*W	1	0.15	0.0738	1.2
G*C*W	2	0.08	0.1920	0.7
Error	24	0.04		17.2
Model R^2		**0.8849**	**<0.0001**	

Data are mean squares (MS) and probabilities for an effect caused by random (*P*) of a three-way fixed effects analysis of variance across species. ω^2 gives the relative 'contribution' of each factor to the total variation (see Methods). Factor levels: grassland type (G; dry, mesic, wet), clipping treatment (C; control, above-ground vegetation removed) and water addition (W; control, 1L of water added every second day, see Methods) on the final proportion of surviving seedlings (arcsine transformed). Significant effects are given in bold.

Results of the survival analysis showed that cumulative seedling survival was significantly higher in wet grassland (59 of 60 plants, i.e. 98.3% at the end of the experiment) than in mesic (40 of 60, i.e. 66.7%) and dry grassland (36 of 60, i.e. 60.0%) and that survival on plots receiving extra water (87 of 90 plants, i.e. 96.7% at the end of the experiment) was significantly higher than on non-watered plots (48 of 90, i.e. 53.3%; Fig. 2.1). In contrast, across the other treatments there were no significant differences in survival among the study species (69.6, 82.5 and 70.6% in *V. elatior*, *V. pumila* and *V. stagnina*) or between clipped (73.3%) and un-clipped (75.6%) plots.

Analysis of variance revealed significant effects of grassland type (G), water-addition (W) and the G*W interaction on the proportion of surviving seedlings across species, while clipping (C) had no effects (Table 2.2, Fig. 2.2a). Together, water-addition, grassland type (i.e. soil water availability) and their interaction accounted for almost 80% of the total model variation. In the dry grassland, without water-addition significantly more seedlings survived below the surrounding vegetation than on clipped plots (Fig. 2.2a; 0.467 vs. 0.067; contrast analysis: $F_{1,24}$= 11.99, p=0.0020).

Analysis of relative neighbour effects (RNE) indicated that the canopy had significantly positive effects in dry grassland plots while the effects on survival were neutral or slightly positive in mesic and wet grassland (Fig. 2.3).

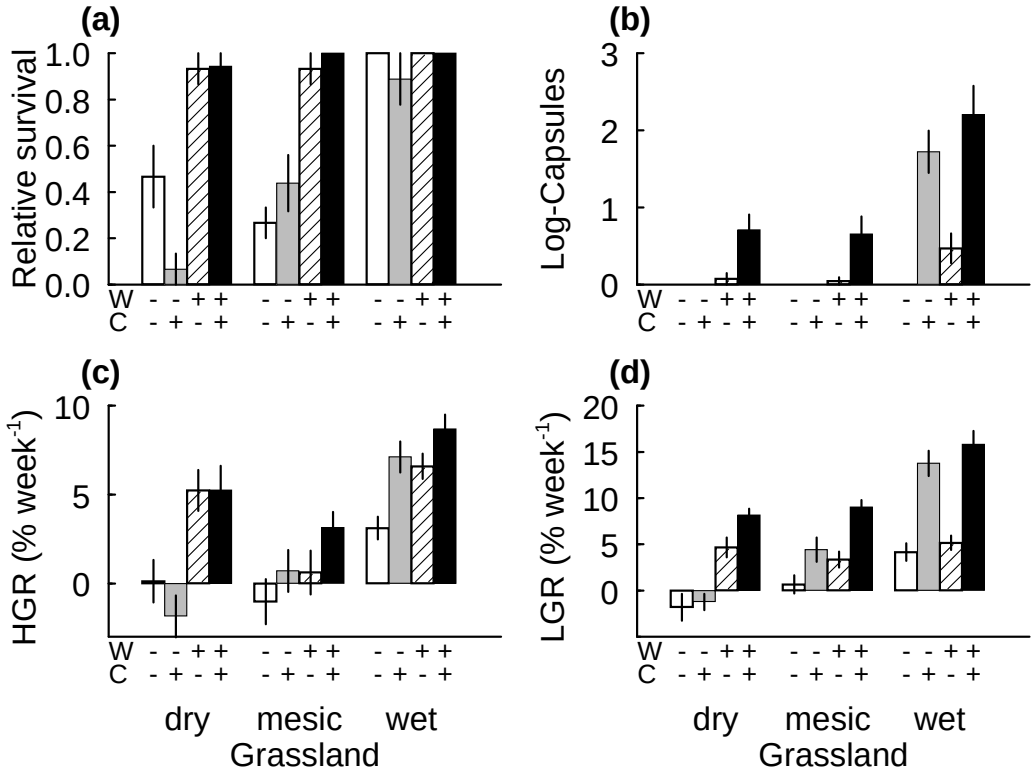

Figure 2.2 Mean relative survival (proportion) of seedlings at the end of the experiment (a), average number of capsules (Log_e-transformed) (b), average height growth rate (HGR, % $week^{-1}$) (c) and leaf number growth rate (LGR, % $week^{-1}$) (d) across species in dry, mesic and wet grassland. Surrounding vegetation on plots was clipped (C+) or not (C–) and plots received additional watering (W+) or not (W–). Treatment combinations are indicated below the x-axis. Data are means ± SE (n=3 for survival and 15 for capsule production, HGR and LGR).

Growth

Since I used relative rates for height growth and leaf accumulation, average relative rates of some treatment combinations were zero or even negative (Fig. 2.2). This means that there was a net decrease in height and leaf number with time in some individuals. Negative rates were not only found in plants that died but also in individuals that survived throughout the experiment.

While differences in HGR between clipped and unmown plots were insignificant in the dry grassland, clipping increased HGR and LGR in the mesic and wet grassland (Fig.

2.2c., d.). The effect of clipping on LGR increased with water availability (grassland type). In contrast, water-addition had the largest effects on both HGR and LGR in the dry grassland, while effects in the wet grassland were small (Fig. 2.2c., d.).

Soil water availability (i.e. grassland type) accounted for the largest proportion of the total variance in HGR and LGR (Table 2.3). Water-addition and species effects accounted for 12 and 8% of the variation in HGR, while clipping (17%) and water-addition (12%) had the largest effects after grassland type on LGR. There were no significant differences among species in LGR. The significant G*S*C interaction on LGR (Table 2.3) was owing to a larger response of species to clipping in mesic and wet grassland than in the dry grassland (significant G*C effect) and to a larger LGR of *V. stagnina* in the mesic grassland (significant G*S effect), since the response to clipping did not differ significantly among species across grassland types (non-significant S*C effect, Table 2.3).

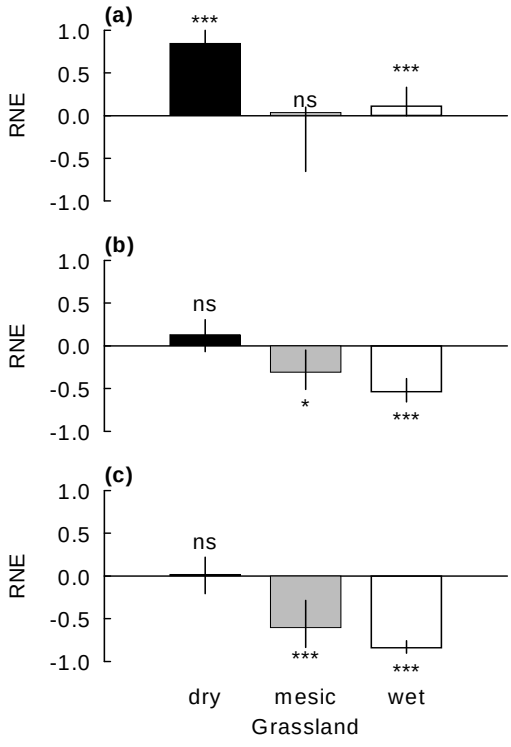

Figure 2.3 Relative neighbour effects on relative survival (a), relative height growth (b) and relative leaf accumulation (c) across species in dry (black bars), mesic (shades bars) and wet (white bars) grassland. Data are means with 95% confidence intervals from a Monte-Carlo analysis (bootstrap sample size: 10000). *P*-values (ns, p>0.05; *, p<0.05; ***, p<0.001) were based on the obtained bootstrap distribution and indicate significant deviations from zero.

Across species and grasslands, water-addition led to higher HGR and LGR, and the positive effect of clipping of the surrounding vegetation on HGR and LGR did not depend on water-addition (non-significant C*W interaction, Table 2.3).

Relative neighbour effects (RNE) of the canopy were significantly negative in terms of relative height and leaf accumulation in mesic and wet grassland plots, while in dry grassland the effects were not significantly different from zero (Fig. 2.3).

Table 2.3 Effects of grassland type (G), species (S), clipping (C) and water-addition on the height growth rate (HGR), leaf growth rate (LGR) and number of capsules (CAPS) of three violet species.

		HGR			**LGR**			**CAPS**		
Source of variation	d.f.	MS	*P*	ω^2	MS	*P*	ω^2	MS	*P*	ω^2
Intercept	1	**1802.5**	**<0.0001**		**5157.2**	**<0.0001**		**38.20**	**<0.0001**	
Grassland type (G)	2	**482.5**	**<0.0001**	19.0	**759.5**	**<0.0001**	19.9	**13.68**	**<0.0001**	17.2
Species (S)	2	**204.5**	**<0.0001**	7.7	45.8	0.0544	0.8	**3.88**	**<0.0001**	4.6
Clipping (C)	1	**99.6**	**0.0101**	1.7	**1290.4**	**<0.0001**	17.0	**23.76**	**<0.0001**	15.0
Water addition (W)	1	**580.2**	**<0.0001**	11.5	**891.6**	**<0.0001**	11.7	**8.50**	**<0.0001**	5.3
G * S	4	14.0	0.4335	0.0	**51.9**	**0.0114**	1.9	**3.48**	**<0.0001**	8.2
G * C	2	**72.9**	**0.0081**	2.4	**211.2**	**<0.0001**	5.2	**7.80**	**<0.0001**	9.6
S * C	2	13.9	0.3891	0.0	32.0	0.1291	0.4	**1.60**	**0.0033**	1.7
G * W	2	**68.6**	**0.0107**	2.2	**132.2**	**0.0003**	3.1	0.21	0.4682	0.0
S * W	2	12.0	0.4435	0.0	21.0	0.2591	0.1	0.45	0.1925	0.2
C * W	1	2.1	0.7071	0.0	53.4	0.0647	0.5	**2.59**	**0.0023**	1.5
G * S * C	4	6.3	0.7881	0.0	**50.7**	**0.0129**	1.9	**1.47**	**0.0004**	3.1
G * S * W	4	10.8	0.5700	0.0	20.6	0.2591	0.3	**1.13**	**0.0031**	2.2
G * C * W	2	10.4	0.4940	0.0	1.5	0.9091	0.0	0.21	0.4587	0.0
S * C * W	2	6.0	0.6631	0.0	5.0	0.7242	0.0	0.008	0.9681	0.0
G * S * C * W	4	36.3	0.0465	1.8	17.0	0.3565	0.0	0.43	0.1792	0.4
Error	144	14.6		53.6	15.4		37.0	0.27		31.0
Model R^2		**0.5630**	**<0.0001**		**0.7164**	**<0.0001**		**0.7655**	**<0.0001**	

Data are results of a four-way general linear models analysis of variance. Factor levels: grassland type (G; dry, mesic, wet), species (S; *Viola elatior*, *V. pumila*, *V. stagnina*), clipping (C; control, above-ground vegetation removed) and water addition (W; control, 1L of water added every second day, see Methods). ω^2 gives the relative 'contribution' of each factor to the total variation (see Methods). Height growth rate (HGR), and leaf number growth rate (LGR) were expressed in % $week^{-1}$. Significance threshold after Bonferroni correction for three tests: 0.0167. Significant effects are given in bold.

Reproduction

In contrast to survival and height growth, water-addition alone was not sufficient for production of seed capsules (Fig. 2.2b). In the dry and mesic grassland, plants receiving additional water only produced capsules on clipped plots. In the wet grassland, capsule production was higher on clipped than on unmown plots. Plants that did not receive extra water produced significantly more capsules when the surrounding vegetation was clipped (grey bars, Fig. 2.2b) than plants on unmown, watered plots (hatched bars) and nearly as many capsules than plants on clipped, watered plots (black bars). These relationships resulted in a significant C*W interaction (Table 2.3). Consequently, G and C accounted for equally large proportions of the explained variance (17 and 15%), while the main effect of water-addition was responsible for only 5% of the variation (Table 2.3).

There were some higher order interactions on the number of seed capsules. The G*S*W interaction probably was related to a much higher capsule production of *V. pumila* and *V. stagnina* in the wet grassland (G*S interaction), since the response to water-addition did not vary significantly among grassland types or species (non-significant G*W and S*W interaction). Also the significant G*S*C interaction in the end was related to higher reproductive allocation of *V. pumila* and especially *V. stagnina*, that led to higher numbers of capsules on plots of the wet grassland (G*S), on clipped plots (S*C) and the larger response to clipping in wet grassland (G*C).

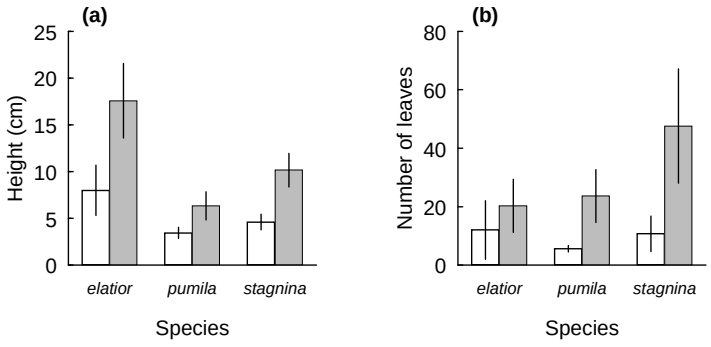

Figure 2.4 Height (a) and number of leaves (b) of vegetative (white bars) and reproductive (grey bars) individuals of *Viola elatior*, *V. pumila* and *V. stagnina* surviving until the end of the experiment across treatments. Data are means ± 95% confidence intervals. Sample sizes were 32 and 7 vegetative and reproductive individuals in *V. elatior*, respectively, 30 and 22 plants in *V. pumila* and 25 and 19 individuals in *V. stagnina*.

Reproduction of those individuals that survived until the end of the experiment was significantly related to height in all three species (Fig. 2.4a.). Non-reproductive individuals of *V. pumila* and *V. stagnina* also had significantly lower numbers of leaves than individuals producing at least one capsule, while this difference was not significant in *V. elatior* (Fig. 2.4b).

Environmental effects of treatments

The study year was exceptionally warm (deviations of monthly mean temperatures from long-term average (1961-90): +1.7, +3.9, +1.8, +4.4, +0.2° C from May through September) and dry with only 228 mm of precipitation from May through September (deviation of –66 mm as compared to the long-term average). Experimentally watered plots received 30 (May), 140 (June), 130 (July), 120 (August) and 100 mm (September) of extra water. In total (520 mm), this was about twice as much as the natural precipitation during this period. It should be noted, however, that addition of 10 mm of water to the experimental plots does not have the same effect than the same amount of precipitation. Events of natural precipitation will reduce soil and air temperatures and increase air humidity and thus lead to reduced rates of evapotranspiration and water loss in plants (e.g., Larcher 1995). These effects will not be achieved by experimental watering during otherwise dry conditions as in the present experiment. Still experimental water-addition considerably improved the plants' water relations in comparison to control plots, leading to significant effects on vital rates (see below).

Mown plots received between 70 and 100% of incident photosynthetic active radiation (PAR) across measurement occasions and grasslands, while relative illumination in unclipped plots was only between 30 and 60%. PAR levels on unclipped plots decreased considerably between June 13 (occasion 2) and June 30 (occasion 3). This coincided with the period of rapid height growth of the meadow matrix species and canopy closure.

Daily mean temperatures just below soil surface were higher in mown than in control plots. The difference between mown and unmown plots increased during the experiment and was on average about 1-1.5° C. Daily maximum temperatures differed on average by about 4° C and in the most extreme cases up to about 8° C between mown and unmown plots.

Discussion

The experimental results strongly suggest that water availability was of crucial importance for plant survival and growth because (i) cumulative seedling survival was close to complete in wet grassland plots and on watered plots (Fig. 2.1) and (ii) water-addition largely removed differences between grassland types (Figs 2.1, 2.2). Grassland types also differed in other characteristics such as species richness, canopy height or standing crop (Table 2.1) but these differences were apparently overruled by water-addition during the study year. In years with more beneficial conditions for plant growth, the net effect of interspecific interactions at the experimental site may be negative (cf. Greenlee & Callaway 1996; Tielbörger & Kadmon 2000). Lower standing crop and canopy height in mesic grassland was an effect of the dominance of the relatively low productive and low-growing grass *Festuca rubra* (Table 2.1). However, the average net effect of vegetation canopy on light interception of seedlings and soil temperatures did not differ between grassland types. The selection of grassland types according to their presumed water availability together with the experimental results showing that water-addition removed differences between grassland sites, justifies the treatment of grassland type as a fixed factor (Newman *et al.* 1997; Quinn & Keough 2002).

Differences in seedling survival of two biennial species of sand dune areas between watered and control plots increased from alder-thickets to open dune vegetation (De Jong & Klinkhamer 1988); seedling mortality was significantly correlated with soil water content and increased strongly at soil water contents below 5% soil d.wt. During a prolonged drought period, soil water contents below this level were found in plots of the dry and mesic grassland, while in the wet grassland soil water content was still close to 10% of soil d.wt. (Table 2.1).

The positive 'nurse-plant' effect on water relations that has been demonstrated in dry habitats (e.g., De Jong & Klinkhamer 1988; Holmgren *et al.* 1997; Tielbörger & Kadmon 2000 and references therein) may be directly related to increased soil water content (De Jong & Klinkhamer 1988; but see Nobel 1989). However, my soil temperature recordings (not shown) suggest that it may also be a consequence of reduced thermal stress under a vegetation canopy (cf. Larcher 1995).

Based on a hypothesised trade-off between drought and shade tolerance in plants (Smith & Huston 1989), facilitation may occur, if the amendment of water conditions

with shade exceeds the increased moisture requirements related to the acclimation to shade (Holmgren *et al.* 1997).

I found net positive effect of interspecific interactions on seedling survival on non-watered plots in the dry grassland (Figs 2.2, 2.3). Apparently, in the mesic and wet grassland, the improvement of water conditions through the vegetation canopy could not outweigh negative effects through shading and thus the balance tipped towards net negative effects. In a perennial shrub from a xeric ecosystem, shade always increased HGR while the effects on seedling survival depended on the marginal conditions and varied between seasons (Hastwell & Facelli 2003), suggesting that the effects of interspecific interactions can vary between vital rates. In an experiment on the effects of soil moisture, vegetation canopy, litter accumulation and seed addition on plant diversity at Wicken Fen, Xiong *et al.* (2003) found that at a low elevation site (high soil moisture) germination was higher after seed-addition when the vegetation canopy was removed than in control plots, while there was no difference at a drier site, indicating that positive and negative effects of a vegetation canopy cancelled each other out.

On non-watered plots of the dry grassland also HGR of seedlings was higher under a vegetation canopy than on clipped plots (Fig. 2.2), but this difference was not statistically significant (Fig. 2.3). So here positive and negative effects cancelled out each other. Consequently, the magnitude of effect (ω^2) of the water-addition treatment, of grassland type (i.e. soil water availability) and their interaction explained 78% of the model variation for survival and 33% for HGR (Tables 2.2, 2.3). These values of ω^2 can not directly be compared between analyses or among studies, because, if total variability varies between experiments, ω^2 relates to different relative baselines (Graham & Edwards 2001). However, the relative ranking of ω^2 or the ratio between magnitudes of effect (Underwood & Petraitis 1993) in different analyses or experiments still point out the most important factors. The ratios of the main effects of grassland type and water-addition to the effects of clipping were thus 238 and 407, respectively, for survival and still 11.0 and 6.7 for HGR, while grassland type and clipping were equally important for LGR and water-addition even less important than clipping for reproduction (Tables 2.2, 2.3).

In the Wicken Fen experiment (Xiong *et al.* 2003), two- and three-way interaction explained a large part (40-50%) of the total variation. Analysis of the present experiment revealed only relatively few significant interactions. Since the relative contribution of

interactions to model variation was low (Tables 2.2, 2.3), (i) the interpretation of main effects was relatively straightforward (cf. Underwood & Petraitis 1993; Graham & Edwards 2001), (ii) the importance of main factors, although not statistically additive, was relatively high and (iii) their differential effects on the dependent variables could be evaluated.

In contrast to survival and height growth, competition for light was more important for leaf accumulation rate and reproduction, i.e. clipping of the surrounding vegetation accounted for 15-17% of the total model variation (Table 2.3). A direct comparison of survival and reproduction (Fig. 2.2a, b) further proves that treatment effects on these vital rates differed noticeably. While nearly all seedlings survived after water-addition (Fig. 2.2a), reproductive plants were almost exclusively found on clipped plots (Fig. 2.2b), and also on non-watered plots in the wet grassland, capsule production only occurred after removal of the vegetation canopy. Leaf accumulation rate and reproduction decreased under vegetation canopy due to lower rates of carbon gain under shady conditions. Competition for light triggered reproductive output also in other species of the genus *Viola* (Mattila & Salonen 1995; Jensen & Meyer 2001; Moora *et al.* 2004), indicating that at least the species from semi-natural habitats are light demanding for successful sexual reproduction. Similarly, mowing and the removal of litter, which both reduce competition for light, increase the performance of single herb species in terms of growth and reproduction and positively influence species richness in grasslands (e.g., Lepš 1999; Jensen & Meyer 2001; Lennartsson & Oostermeijer 2001). This highlights the effects of management for biodiversity and community structure in semi-natural habitats (e.g., Oostermeijer *et al.* 1994a; Lennartsson & Oostermeijer 2001; Eckstein *et al.* 2004). In a study with seedlings of fen species, light intensity had larger effects on growth rate and allocation than groundwater level (Kotowski *et al.* 2001), which probably was related to the fact that the use of fen peat in this experiment only lead to moderate levels of water stress.

Logistic regression (not shown) demonstrated that there was a threshold size for reproduction in terms of size in the study species. For a 50% probability of reproduction *V. elatior* had to reach a height of 22 cm, *V. pumila* needed 10.8 leaves or a height of 5 cm and *V. stagnina* 25.7 leaves or a height of 7.4 cm. Consequently, flowering and vegetative plants differed significantly in height and leaf number (except for *V. elatior*; Fig. 2.4), which shows that photosynthetic carbon gain and reproduction are closely linked (e.g.,

Solbrig *et al.* 1988; Mendéz & Obeso 1993; Mattila & Salonen 1995; Schmid *et al.* 1995; Jensen & Meyer 2001; Mendéz & Karlsson 2004). However, the fact that both leaf number (that was influences by clipping and water availability) and height (that was affected by water availability and water-addition) were closely related to reproduction underpins the complex relationships between environmental factors, biotic interactions and plant performance.

There is now an increasing body of evidence concerning positive plant-plant interactions. It appears that facilitation does not only occur in habitats characterised by extreme environmental conditions but that the net outcome of interactions always depends on the balance between facilitation and competition (e.g., Holmgren *et al.* 1997; Brooker & Callaghan 1998; Bruno *et al.* 2003). However, the strength of facilitation increases with environmental stress (Fig. 2.2; cf. Callaway *et al.* 2002) and varies with season and climatic conditions (Tielbörger & Kadmon 2000; Hastwell & Facelli 2003).

My data suggest that by ameliorating environmental stress, facilitation through other plants may - in the short term - reduce the strength of environmental controls and, consequently, increase the realized niche of species (cf. Bruno *et al.* 2003). For example, under the canopy of grasses, all three violets established on sandy soil in dry grassland plots, while their natural habitat are floodplain grasslands with strongly fluctuating water tables (Hölzel 2003). However, whether facilitation will lead to the establishment of species depends on the importance of facilitation and environmental control on all stages of the life-cycle and the resulting importance of the vital rates for population growth rate (Caswell 2001). While there were positive effects of the canopy on survival in the present study, leaf growth and reproduction were reduced by competitive effects (Figs 2.2, 2.3). The balance between facilitation and competition thus varies among life-cycle stages as also shown by Rousset & Lepart (2000) and Hastwell & Facelli (2003). There seem to be no large differences between the responses of woody vs. herbaceous species to facilitation at least as regards the seedling and juvenile stages (De Jong & Klinkhamer 1988; Callaway & Walker 1997; Rousset & Lepart 2000; Callaway *et al.* 2002; Hastwell & Facelli 2003; this study). However, with respect to the effects of litter as an agent of 'after-death-interactions' (Facelli & Facelli 1993) there may be important differences between ecosystems. In a meta-analysis Xiong & Nilsson (1999) showed that the effects of grass-litter in contrast to other litter types and the effects of litter in grasslands systems in contrast to other ecosystems could be positive or negative. This

was confirmed in an empirical study with eight floodplain herbs that demonstrated a unimodal response of germination and strongly positive effects of litter on germination of *V. elatior* and *V. pumila* under dry conditions (Eckstein & Donath, unpubl.). Whether this is a consequence of co-evolution in grassland systems or also applies to other ecosystems remains to be examined.

In summary, the present study has shown that environmental conditions and biotic interactions may have differential effects on different vital rates, and that the finely tuned balance between positive and negative interspecific interactions may also affect plant survival, growth and reproduction in temperate habitats. These findings further our understanding on interspecific interactions and highlight the complex interplay between spatial and temporal environmental variation and biotic interactions in structuring plant communities (Silvertown *et al.* 1999; Bruno *et al.* 2003; Lortie *et al.* 2004).

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Chapter 3

The effects of management and environmental variation on population stage structure in three river-corridor violets

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Abstract

Population stage structure of plants, i.e. the density and frequency of individuals in different stages of the life-cycle, is a crucial aspect of population viability that depends on a variety of factors. In this paper, we evaluated the effects of (i) management and year, (ii) location (population) and time (year) and (iii) of local habitat quality and population factors on population stage structure of three morphologically similar, closely related violets from floodplains, *Viola elatior*, *V. pumila* and *V. stagnina*.

We hypothesized that owing to similar life-cycles there should be no significant differences in population stage structure among species. We analysed population stage structure in managed vs. abandoned populations to test whether a proposed effect of management acts through the creation of regeneration niches. We further tried to identify which habitat factors are responsible for possible management effects.

We established permanent plots (0.25 m^2) in 27 populations of the species in two different regions (Rhine floodplains, Germany; Dyje River floodplains, Czech Republic) and recorded frequency and density of seedlings, small and large vegetative plants and small and large flowering plants during two years.

There were significant differences among species, indicating that the species have different life histories. Furthermore, there was a significant effect of management on population stage structure in two of the species. Management significantly increased the proportion of seedlings, over and above possible differences between regions. In our data set, the effects of spatial variation among populations were generally larger than

the effects of temporal variation. The only factor that affected the density of life-cycle stages was the cover of bryophytes, while the cover of higher plants, litter or soil (local habitat quality), or isolation and population size (population factors) had no effects.

Introduction

The growth and viability of populations is ultimately determined by year-to-year transitions of individuals between the stages of the life-cycle (Caswell 2001). According to their population stage structure, i.e. the frequency and/or density of life-cycle stages with different contributions to the organism's vital rates, several types of populations can be recognised in both plants (e.g., Rabotnov 1985) and animals, including humans. In plants three main types of populations have been identified (Oostermeijer *et al.* 1994a; Hegland *et al.* 2001), (i) dynamic populations, characterised by a large proportion of young individuals (seedlings, juveniles), (ii) stable populations, with a higher proportion of adult plants but still a considerable percentage of young individuals, and (iii) regressive populations, in which adult life-cycle stages dominate and recruitment of new individuals is impeded. Regressive populations are currently of great concern in evolutionary biology, conservation biology, and management (Oostermeijer *et al.* 1994a; Bühler & Schmid 2001; Hegland *et al.* 2001) since habitat destruction and fragmentation may cause deterministic species extinctions (Tilman *et al.* 1994), which can be delayed due to the persistence of adult perennial plant individuals. Analysing population stage structure is a useful tool for the study of population viability if long-term demographic data for the species in question are lacking. This approach has successfully been applied in conservation biology to assess the population viability and to develop management strategies of rare plants (e.g., Oostermeijer *et al.* 1994a; Hegland *et al.* 2001) or indicator species (Bühler & Schmid 2001). Additionally, population stage structure has been used to describe successional stage and population viability in studies of seedling establishment (Morgan 1997), population genetics (Schmidt & Jensen 2000), and species invasion (Dietz *et al.* 1999).

Population stage structure may depend on the specific life-cycle of the species in question (e.g., Silvertown *et al.* 1993) and shows temporal and spatial variation (e.g., Svensson *et al.* 1993; Horvitz & Schemske 1995; Riba *et al.* 2002). This variation may be related to local habitat quality, such as the productivity and cover of the vegetation, or population factors, such as the degree of isolation and population size. Especially

species from meadows depend on management (Lennartsson & Oostermeijer 2001) which reduces competition for light and thus creates suitable habitat conditions for many herbs.

The objectives of the present paper were, first, to compare and evaluate the importance of management, species, location, and time on population stage structure of three morphologically similar, closely related, endangered species of floodplain violets. Secondly, we tried to identify habitat factors that are responsible for differences in population stage structure across species.

We studied the following predictions/questions:

(1) Since the species are similar in morphology, closely related and all occupy semi-natural floodplain grasslands, we were interested in testing whether these species show a similar life-cycle. If they do, we would expect that their population stage structure should not differ significantly.

(2) Since the species exclusively occur in semi-natural habitats, management should have a strong effect on population stage structure. If the effect of management is mainly through the creation of regeneration niches, we would predict that the number of seedlings should be significantly higher in managed vs. unmanaged populations.

(3) In case that management has a strong effect on population stage structure, we tried to identify the local environmental factor, such as the cover of higher plants, litter, bryophytes, or soil, that may be responsible for the management effect,. We directly compared the effects of local habitat quality and population factors on density of life-cycle stages.

(4) We further asked whether spatial or temporal variation has a larger effect on population stage structure of the study species.

Materials and Methods

Study species

The study species are perennial iteroparous hemicryptophytes of the genus *Viola* and belong to the section *Viola*, subsect. *Rostratae* (Kirschner & Skalický 1990; Quinger 1993) and are morphologically similar (Hegi 1975; Quinger 1993; Rothmaler 1994). The taxonomy and nomenclature of vascular plants follow Rothmaler (1994). In Central

Europe all three species show affinity to the valleys of large lowland rivers, which is yet a poorly understood distribution pattern (Burkart 2001). All three species are endangered across Central Europe where they reach their western distribution limits (Schnittler & Günther 1999).

Viola elatior is the tallest of the three species, reaching a maximum height of about 80 cm. It has a Western Eurasian-continental distribution with its core ranging from Siberia to eastern Europe (Hultén & Fries 1986). Its westernmost populations are known from France (Didier & Royer 1988). *Viola elatior* occurs mainly along sunny scrubby woodland fringes adjacent to floodplain meadows and on clearings of alluvial forests.

Viola pumila has a similar distribution pattern as the former species, reaching its westernmost limit along the Rhine (Hultén & Fries 1986). The species has a maximum height of about 50 cm. *Viola pumila* occurs mainly on species-rich semi-natural continental meadows of the alliance *Cnidion venosi* Balátová-Tuláčková 1965, which are mown once or twice a year.

Viola stagnina (syn. *V. persicifolia*) also has a Western Eurasian distribution range, reaching even the hemiboreal vegetation zone in Scandinavia (Hultén & Fries 1986). With a maximum height of 30-40 cm, it is the smallest of the three study species. It occurs in the wetter parts of continental meadows of the alliance *Cnidion*, as well as in *Molinion* or *Calthion* meadows. It seems to be sensitive to severe summer drought. In Central Europe, *V. stagnina* is not strictly bound to floodplains and also occurs in fen meadows, tall-sedge swamps and along ditches (e.g., Pullin & Woodell 1987; Quinger 1993).

Design of study sites and permanent plots

The study was carried out in two regions, viz. along the Upper Rhine (Germany) and in the floodplains of the lower Dyje River (Czech Republic), representing the two strongholds of the study species in Central Europe (Hölzel 2003). The German populations are at the western edge of species ranges, while the Czech populations are closer to the main distribution ranges. The populations of the species studied were scattered over an area of about 370 km^2 within the Rhine floodplains and of about 350 km^2 in the Dyje floodplains. Extant populations of *V. stagnina* in the Upper Rhine region are scattered over an area of appr. 60 x 25 km. Only three populations of *V. stagnina* occur within the Rhine valley, the bulk of populations is found in the Lower Main area

south of Frankfurt. The regional climate is sub-continental in the Upper Rhine area with an annual mean temperature of 10.4 °C and 607 mm of precipitation. Soils within the floodplain can be classified as alluvial Gley types (Hölzel 1999). The region is densely populated and the landscape fragmented through settlements, roads and large areas of intensive agricultural production. The ground water level varies considerably across the Upper Rhine area, with large fluctuations close to the river Rhine and small fluctuations in the Lower Main area. Only few of the violet populations are subject to direct flooding of the recent floodplain. Most of the sites belong to the hybrid or fossil floodplain and will be submerged through ascending groundwater during short periods of high water levels (Hölzel & Otte 2001). The regional climate of the Dyje floodplains north-west and south of Břeclav (annual difference in monthly mean temperatures: 21 °C) is more continental than in the Upper Rhine area (18 °C). The Dyje area has an annual mean temperature of 9.2 °C and 480 mm of precipitation. Alluvial Gley soils predominate within the floodplain (Grulich *et al.* 2000). North-west of Břeclav, intensive crop fields prevail outside the floodplain, whereas directly in the floodplain, forests and some relatively un-intensively managed meadows have been preserved. The sub-region south of Břeclav is only sparsely populated and here, the largest proportion of land (84%) is covered with forests (Grulich *et al.* 2000), surrounding large and middle sized patches of alluvial meadows. Before the river canalisation in 1972 our study sites were regularly flooded. The dynamics of ground water were preserved after canalisation, with its maximum (about 40 cm below the surface) in late April and minimum (185 cm below the surface) in mid-August (Prax *et al.* 1997).

Within each region, we selected three (*V. stagnina*), five (*V. pumila*) and five (*V. elatior*, Dyje) to six (*V. elatior*, Rhine) populations that represented the characteristic vegetation types: the populations of *V. pumila* and *V. stagnina* were situated on extensively used continental meadows mown once or, rarely, twice a year. The populations of *V. elatior* were confined to ecotonal habitats, such as fringes and openings/clearings of alluvial forests adjacent to floodplain meadows. Stands of the study species were sometimes only separated by some ten metres. A line transect was laid out within each population, and depending on population size and expansion, three to eight permanent plots of 50 cm x 50 cm (0.25 m^2) containing individuals of the study species were established. The plots were selected to represent the variation in shoot density present across populations. In total, we investigated 1900 individuals in 122 plots from 27 populations.

Life-cycle stages

All three species have a long-term persistent seed bank, which may remain viable for several years (Moore 1983; Rowell 1983 as cited in Pullin & Woodell 1987; Schopp-Guth 1997; Hölzel & Otte 2001). Seeds germinate in spring, and seedlings develop a few leaves by the end of summer. During May to early June generative plants may bear one to several open-pollinated (chasmogamous) flowers, which are potentially cross-pollinated and mature into capsules from early June to early July. Later in the season, cleistogamous flowers, which remain closed and are obligatorily self-pollinated, may be formed by the same individual. The life-cycle stages ("age states", Rabotnov 1985) used in the present paper were based on the presence of flowers and the number of shoots:

- **Seedlings** (**s**), defined by the presence of cotyledons,
- **Small vegetative** (**v1**), non-flowering adults plants, with one shoot,
- **Large vegetative** (**v1**), non-flowering adult plants with two or more shoots,
- **Small generative** (**g1**), flowering plants with one or two shoots,
- **Large generative** (**g2**), flowering plants with three or more shoots.

In each permanent plot all individual plants were recorded with their coordinates. We use the term "individual" in the sense of supposed "independent physiological units". With the density of shoots encountered in the field, we were able to assign each shoot to a particular single- or multiple-shoot plant. This approach was verified by careful excavation of a few multiple-shoot plants outside our plots. Within each population, i.e. across all plots, we calculated the frequency of each life-cycle stage. For each population, we also estimated the mean density of each life-cycle stage per m^2. We visually estimated the cover (%) of vascular plants, bryophytes, litter, and bare soil per plot. In the analyses we used the calculated mean cover of vascular plants, bryophytes, litter, and bare soil across all plots for each population. Population size (adults plants) was estimated on a logarithmic scale, i.e. some tens (=1), some hundreds (=2) and some thousands of plants (=3). Management was scored for each population using information on management contracts and field observations on actual management as regular management (i.e. mown once a year) or irregular (no mowing during the past years). A few populations of *V. elatior* along paths were only mulched, i.e. the mown material was not removed. However, we did not differentiate between management

types, since we considered these different managements to have similar functional effects, i.e. reduction of light competition and creation of gaps in the closed meadow vegetation.

Distance measurements

Using digital maps, we determined the geographic coordinates of all known extant populations with an accuracy of roughly 50 m. Then we calculated the linear distance between all possible pairs of populations for each species-region combination. We used the ln-transformed distances in this half-square matrix to compare the frequency distribution of distance classes between regions. From the distance matrix, we recorded for each population the distance to the closest population as a measure of isolation.

Statistics

To study the effects of the species, region, and year on the density of plants of different life-cycle-stages, we carried out a fixed factor three-way repeated measure analysis of variance (von Ende 1993) with 'time' as the repeated-measures (within-subjects) factor. Since the within-subjects factor only had two levels and the assumptions of 'circularity' and 'compound symmetry' did not matter (cf. von Ende 1993), we used the univariate ANOVA results.

To evaluate the importance of habitat quality vs. population factors for the density of plants of all life-cycle-stage, we used a multivariate general regression model (GRM) with forward stepwise selection of variables, which considered the following terms: cover (%) of vascular plants, bryophytes, litter, and bare soil (local habitat quality), isolation (km) and population size (ln-scale; both population factors) as continuous variables, and year as a categorical variable. For this analysis three populations were omitted. In two populations there were no data on local factors, and one population of *V. stagnina* represented an outlier by visual inspection of the data, that probably was due to an error in cover estimation.

Differences in the distribution of pairwise distances between regions were tested for each species separately, using the non-parametric Wald-Wolfowitz test (Siegel & Castellan 1988).

Frequency data were analysed using log-linear analysis for contingency tables (Quinn & Keough 2002). Since the data structure did not allow evaluation of all factors

simultaneously, three different log-linear analyses on the frequency of life-cycle-stages were carried out. In the first analysis we tested the whole data set for the effects of *species* (*Viola elatior*, *V. pumila*, *V. stagnina*), *year* (2001, 2002) and *region* (Dyje [R1], Rhine [R2]) as design factors on the frequency of *life-cycle stages* (s, v1, v2, g1, g2). Secondly, we tested the effects of *year* and *management* (irregular [M0], regular [M1]) on frequency of *life-cycle stages* for each species separately. Thirdly, since the species did not occur within the same populations, we compared for each species the effects of spatial (i.e. among populations) and temporal (i.e. between years) variation on stage frequency.

However, since it became apparent during the study that all populations within the Rhine region (R2) were regularly managed, while within the Dyje floodplain (R1) some populations were managed and others abandoned since some years, the effect of management could not easily be separated from the possible effect of region. Therefore, to examine whether there is an effect of management (M1 vs. M0) above the effects of region, we applied a Monte Carlo simulation (cf. Manly 1991) using the program 'Poptools' (version 2.55) developed by G. Hood (cf. www.cse.csiro.au/poptools/index.htm, viewed 2003-06-27). We expressed the effect size of region and management by the ratio of individuals of different life-cycle stages between R2 and R1 and the ratio of individuals of different life-cycle stages between M1 and M0, respectively.

From our data, we calculated a matrix that contained for each life-cycle stage the probabilities of belonging to one of the management categories (M0, M1) or regions (R1, R2). Using these probabilities and the number of observed individuals in each of the life-cycle stages, we calculated a matrix whose cells contained the random binomial deviation based on the appropriate numbers of individuals and probabilities. We then calculated for each life-cycle stage the ratio of individuals between management categories (ratio M1 : M0) and regions (ratio R2 : R1). Using a Monte Carlo simulation, we finally generated a bootstrap sample (sample size: 2000) for these ratios for each of the life-cycle stages, from which we calculated the bootstrap mean and 95% confidence interval (e.g., Dixon 1993). If the ratio of seedlings in managed vs. abandoned populations was significantly larger than the ratio of seedlings between the Rhine and the Dyje region, this was taken as an indication that there was a significant effect of *management* over and above the possible effect of *region*.

Except otherwise stated all statistical analyses were done using Statistica for Windows (ver. 6.0, StatSoft Inc.).

Results

Distances between populations

Analysis of the geographic distribution of populations showed that distance distributions were very similar in *V. elatior* from Dyje and Rhine. In contrast, extant populations of *V. pumila* and *V. stagnina* were significantly closer to each other in the Dyje floodplains than those in the Rhine area.

Effects on density

The densities of seedlings (s) were significantly higher in populations from the Rhine area (mean ± s.e. [n]; 28 ± 11 [28] plants m^{-2}) across years and species than in the Dyje area (9 ± 3 [26], Table 3.1). Seedling density was higher in 2001 than in 2002 (28 ± 11 [27] and 10 ± 3 [27], significant *year* effect). Populations of *V. stagnina* had higher densities of seedlings, small vegetative plants (v1) and large vegetative plants (v2) than the other two species, though the difference in seedling density was not significant after Bonferroni correction. Despite a non-significant *species*region* interaction, the ranking of species in terms of seedling density differed between regions. Seedling density of *V. elatior* and *V. stagnina* was higher at the Rhine than at the Dyje river, while *V. pumila* had equal seedling densities in both regions. The v1-density was roughly the same in populations of *V. elatior* and *V. stagnina* in both years, while v1-density increased between years in *V. pumila* (*year*species* interaction, Table 3.1). There were no significant effects of *species*, *region*, or *year* on the density of flowering plants (g1, g2; Table 3.2).

Local habitat quality vs. population factors

In the general regression model (GRM) the cover of bryophytes remained as the only factor affecting density of life-cycle stages across species and regions (Wilks lambda= 0.6708, $F_{5, 42}$= 4.1227, p= 0.0039). The results of univariate analyses showed that bryophyte cover had a significant negative effect on seedling density and a positive effect on large vegetative plants (Fig. 3.1). There were no significant effects of other local factors, of *year*, or of *isolation* and *population size* on density.

Table 3.1 Results of a fixed three-way repeated measure ANOVA for the effects of the between-subject factors *species* (*Viola elatior*, *V. pumila*, *V. stagnina*) and *region* (Rhine, Dyje) and the within-subject factor *year* (2001, 2002) on the density of different *life-cycle stages* (s, seedling; v1, small vegetative; v2, large vegetative; g1, small generative; g2, large generative). Densities were ln-transformed to meet the assumptions of analysis of variance. Significance threshold after Bonferroni correction (five tests): 0.010. Significant effects are given in bold.

		s		**v1**		**v2**		**g1**		**g2**	
Source of variation	DF	MS	P	MS	P	MS	P	MS	P	MS	P
Species (S)	2	3.600	0.064	**5.431**	**0.007**	**5.467**	**0.002**	2.239	0.028	4.284	0.032
Region (R)	1	**16.927**	**0.001**	5.334	0.021	1.525	0.149	0.004	0.931	1.345	0.270
S * R	2	5.328	0.021	1.156	0.284	2.096	0.066	0.561	0.360	1.501	0.261
Error	21	1.144		0.865		0.678		0.523		1.048	
Year (Y)	1	**13.449**	**0.004**	0.521	0.130	0.067	0.558	0.715	0.182	0.004	0.924
Y * S	2	2.891	0.139	**1.428**	**0.005**	0.164	0.435	1.211	0.060	0.276	0.500
Y * R	1	0.004	0.959	0.098	0.502	0.324	0.205	0.005	0.908	0.121	0.581
Y * S * R	2	0.433	0.726	0.433	0.152	0.104	0.587	0.523	0.271	0.070	0.835
Error	21	1.332		0.210		0.189		0.376		0.385	

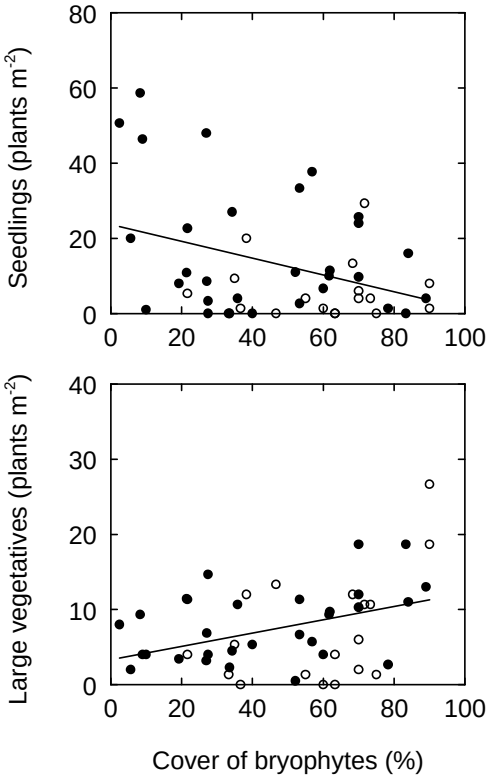

Figure 3.1 Density of seedlings (plants m^{-2}; upper panel) and large vegetative plants (lower panel) in managed and abandoned populations (filled and open circles, respectively) as related to the mean cover of bryophytes (%). Regression slopes (±SE): seedlings (–0.224 ±0.084); large vegetatives (0.089 ±0.032).

Effects on frequency

The effects of various factors on the frequency of different life-cycle stages were analysed using three log-linear analyses. The first log-linear analyses showed that across populations there were significant effects of *species*, *year*, and *region* on the population

stage structure (results not shown). All effects of the log-linear model were highly significant, indicating that there were complex interactions between the studied factors on population stage structure.

The second log-linear analysis showed that *management* had no significant effect on the frequency of life-cycle stages in *V. pumila*, while the other two species showed highly significant effects (Table 3.2) even when the effect of *year* was accounted for in the model. In *V. pumila* there was a significant interaction between *year* and *management* that was owing to the fact that managed populations contained 30 times as many seedlings as abandoned populations in 2001, but this ratio was only 2.6 in 2002.

Table 3.2 Log-linear analysis of *year* (Y, 2001, 2002) and *management* (M, regular, irregular) on the frequency of *life-cycle stages* (L, seedling, small vegetative, large vegetative, small generative, large generative) of *Viola elatior*, *V. pumila* and *V. stagnina*. Chi^2 and DF values (italics) in the Table are the delta Chi^2 and delta DF between the appropriate null-model and the tested model. The term, e.g. YM includes all lower level factors interactions (i.e. Y*M, Y, M), the term M(|Y) denotes the effect of *management* when the factor *year* is already included into the model, i.e. an effect above that of factor *year*; and Y*M denotes the interaction effect of *year* and *management*.

		V. elatior		*V. pumila*		*V. stagnina*	
Model	DF	Chi^2	*P*	Chi^2	*P*	Chi^2	*P*
YM, L	12	42.85		63.64		354.70	
YM, YL	8	21.59		13.3		147.57	
Y	4	21.26	0.0003	50.34	<0.0001	207.13	<0.0001
YM, L	12	42.85		63.64		354.70	
YM, ML	8	26.83		63.14		199.03	
M	4	16.02	0.0030	0.5	0.9735	155.67	<0.0001
YM, YL	8	21.59		13.3		147.57	
YM, YL, ML	4	4.75		12.23		1.51	
M(|Y)	4	16.84	0.0021	1.07	0.8990	146.06	<0.0001
YM, ML	8	26.83		63.14		199.03	
YM, YL, ML	4	4.75		12.23		1.51	
Y(|M)	4	22.08	0.0002	50.91	<0.0001	197.52	<0.0001
YM, YL, ML	4	4.75		12.23		1.51	
YLM	0	0		0		0	
Y*M	4	4.75	0.3139	12.23	0.0157	1.51	0.8249

However, when we summed the number of individuals of each life-cycle stage across years according to management types, managed populations contained 6-90 times more seedlings and between four and seven times more individuals of the other life-cycle

stages than abandoned populations (Fig. 3.2). These ratios were significantly higher for almost all life-cycle stages than the ratios between the numbers of individuals of the two regions (Rhine vs. Dyje). In other words, despite the fact that we observed more individuals in populations of the Rhine area than in the Dyje floodplains, managed populations contained more individuals of the different life-cycle stages than could be explained by the differences between regions. Therefore, management had a significant main effect over and above the effect of regions on population stage structure.

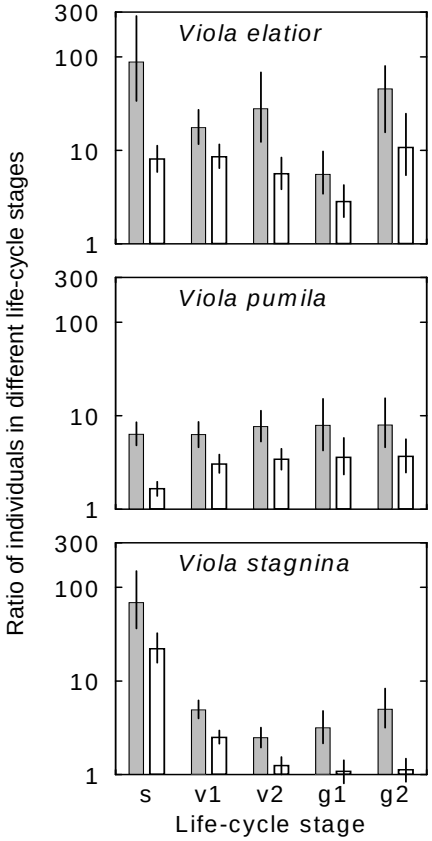

Figure 3.2 Bootstrap means and 95% confidence intervals for *Viola elatior*, *V. pumila* and *V. stagnina* of the ratio between numbers of individuals of certain life-cycle stages in regularly and irregularly managed populations across regions (black bars) and between individuals of certain life-cycle stages in the Rhine region and the Dyje region across management types (white bars). E.g., for *Viola elatior*, the figure shows that seedling number differed by a factor of about 90 between managed and abandoned populations across regions, while the ratio of seedling number between Rhine and Dyje was only about 8 across managements.

The third analysis compared the effects of spatial (among populations) and temporal variation (between years) on the frequency of life-cycle stages for each species separately. It revealed that both *population* (and *year*) had significant effects even when *year* (and *population*) were already included in the model. There was further a significant *population***year* interaction (Table 3.3). However, the model improvement (measured as difference in Chi^2) was always larger when *population* was added to a model than when *year* was added.

Table 3.3 Log-linear analysis of *year* (Y, 2001, 2002) and *population* (P, n=6-11 for each species) on the frequency of *life-cycle stages* (L, seedling, small vegetative, large vegetative, small generative, large generative) of *Viola elatior*, *V. pumila* and *V. stagnina*.

	V. elatior			*V. pumila*			*V. stagnina*		
Model	**Chi2**	**DF**	**p**	**Chi2**	**DF**	**p**	**Chi2**	**DF**	**p**
YP, L	451.12	84		389.12	76		810.68	44	
YP, YL	431.23	80		340.50	72		604.71	40	
Y	19.89	4	0.0005	48.62	4	<0.0001	205.97	4	<0.0001
YP, L	451.12	84		389.12	76		810.68	44	
YP, PL	138.32	44		221.00	40		296.71	24	
P	312.8	40	<0.0001	168.12	36	<0.0001	513.97	20	<0.0001
YP, PL	138.32	44		221.00	40		296.71	24	
YP, PL, YL	128.11	40		167.05	36		155.26	20	
Y(|P)	10.21	4	0.0370	53.95	4	<0.0001	141.45	4	<0.0001
YP, YL	431.23	80		520.54	90		604.71	40	
YP, YL, PL	128.11	40		167.05	36		155.26	20	
P(|Y)	303.12	40	<0.0001	353.49	54	<0.0001	449.45	20	<0.0001
YP, YL, PL	128.11	40		167.05	36		155.26	20	
YPL	0.00	0		0.00	0		0.00	0	
Y*P	128.11	40	<0.0001	167.05	36	<0.0001	155.26	20	<0.0001

Discussion

Frequency and density of life-cycle stages varied between species (significant effect of *species*, cf. Table 3.2) despite similar life-forms, great morphological similarity, and taxonomic relatedness. This indicated that there were species-specific differences in fecundity, survival, and growth of the life-cycle stages, which resulted in different transition probabilities between stages. These differences between population stage structures demonstrate that the species have different life-cycles and may be adapted to different environmental conditions of the occupied habitats (cf. Silvertown *et al.* 1993). Consequently, the temporal changes in density or frequency of stages were not synchronized among species (*species*year* interactions, not shown), and species responded differently to management (Table 3.2). This is in line with a study on the demography of three species of the genus *Pinguicula* where e.g., flowering frequency was not synchronised among years (Svensson *et al.* 1993).

We found evidence for a greater importance of spatial variation (i.e. among populations) than of temporal variation (Table 3.3) for stage frequency, though both factors had significant effects. There were considerable differences in weather conditions between the study years, which may be responsible for the year effect. The first study year had a wet and warm March, which led to a seedling flush, and a very warm and dry summer, whereas the year 2002 was more dry during seedling germination in spring. There were also differences in weather conditions between regions. From March to May there was twice as much precipitation in the Rhine area (181 mm in 2001 and 170 mm in 2002) than in the Dyje floodplains (93 mm and 86 mm), while, in turn, in July and August there was about three times as much precipitation in the Dyje region than in the Rhine region in both years. However, these differences did not result in a significant *year*region* effect on stage density (Table 3.1).

Temporal variation appears to be crucial especially in annual species with population densities varying by several orders of magnitude from year to year (e.g., Grubb 1986). However, also in perennial species there may be large temporal variation (e.g., Riba *et al.* 2002), and patterns of spatiotemporal variability may differ among traits (Horvitz & Schemske 1995).

For our perennial study species, temporal variation between years was lower than spatial variation among populations. This may be caused by differences in management among populations as well as other environmental factors, such as soil fertility, water availability, and/or competition that can be generally summarised as differences in habitat quality (e.g., Riba *et al.* 2002).

Management of the populations through annual mowing or mulching (a few *V. elatior* populations) resulted in higher numbers of individuals in all stages, and thus in higher population densities. Most important were the effects of mowing on the frequency of seedlings across years (Fig. 3.2): managed populations contained 6-90 times more seedlings than abandoned populations did. However, log-linear analysis indicated that the effect of management differed between years in *V. pumila*, with no consistent management effect on population stage structure across years.

The important effect of management found in the present study corresponds to the results of other studies from semi-natural (meadow) habitats that emphasize the importance of management for the viability of populations of meadow species and for species diversity (Ekstam & Forshed 1997; Kotorová & Lepš 1999; Hegland *et al.* 2001;

Lennartsson & Oostermeijer 2001; Jensen & Meyer 2001). However, results of a long-term study (Linusson *et al.* 1998) suggested that even continued land-use may probably not be sufficient for biodiversity conservation and that other effects, such as the atmospheric input of nutrients, may be a threat to plant species richness. Moreover, it has been demonstrated that differences in the effects of various management regimes on population growth of rare plant species may exist (Lennartsson & Oostermeijer 2001). In an experimental study, the rare *V. elatior* established more successful in clipped plots than the common *V. mirabilis*, indicating competition for light as a limiting factor for the former. Management (and historic reasons) were suggested as possible reasons for the low abundance of *V. elatior* in Estonian wooded meadows (Moora *et al.* 2003).

Results from our general regression model that evaluated the effects of population factors vs. habitat quality suggested that only the cover of bryophytes had a significant effect on the density of life-cycle stages (Fig. 3.1). Bryophyte cover tended to be higher in unmanaged populations (58 ± 4.8% [16], mean ± s.e. [n]) than in managed sites (43.7 ± 4.8% [27]), though this difference was only marginally significant ($t = 1.937$, $DF = 41$, $p = 0.059$).

In contrast, other studies in semi-natural grasslands have demonstrated the negative effect of litter accumulation on seedling recruitment and species richness after abandonment (e.g., Jensen & Meyer 2001) and emphasised the importance of litter for structure and function of plant communities (Facelli & Picket 1991; Xiong & Nilsson 1999). The effect of litter may be positive or negative, depending on its amount and structure (Nash Suding & Goldberg 1999).

However, there may also be a strong effect of the bryophyte layer on seedling recruitment (cf. During & van Tooren 1990, and references therein). Since the direction of this effect varied among studies, it has been proposed that the effect of bryophyte cover on germination may be negative in mesic habitats (van Tooren 1990; Špačková *et al.* 1998; Zamfir 2000) and positive in relatively dry habitats (Rusch & Fernández-Palacios 1995; Ryser 1993). However, also for species of dry Alvar-grassland from the Baltic island of Öland, the effect of bryophyte cover on seedling germination has been species-specific (Zamfir 2000). As with litter, the effect of bryophytes may differ (i) between years depending on the actual weather conditions (van Tooren 1990) and (ii) also between ontogenetic stages of the life-cycle as our data suggest (Fig. 3.1). While seedling emergence may be hampered, those individuals that manage to germinate

within the bryophyte layer, may benefit during the establishment phase from e.g., higher soil moisture (e.g., Sohlberg & Bliss 1987, as cited in Zamfir 2000) or protection from insect or rodent herbivores (e.g., During & van Tooren 1990).

The results for the present study emphasize the importance of de-intensive meadow management for the conservation of these endangered species. We have shown that populations within the Rhine floodplains (that all were subject to management contracts) contained more seedlings than population within the Dyje floodplains, and that there was a general effect of management over and above the difference between regions. Our data suggested that the main effect of management was the establishment of regeneration niches (cf. Grubb 1977), probably through the removal of the bryophytes layer and the creation of gaps. Managed populations had much higher densities and numbers of seedlings (Fig. 3.2).

It should be noted, however, that *V. elatior* which occurs in the margins of alluvial forests, has a lower mowing compatibility than the other two, true meadow species (cf. Hölzel 2003; Danihelka, pers. obs.). Additionally, in relatively fertile sites, the violets will probably depend on regular management that will reduce competition, while in nutrient-poor habitats, especially *V. pumila* (and *V. elatior*) may persist for extended periods even under fallow conditions (Hölzel 2003). Efficient management for conservation should take these species- and habitat-specific differences into account.

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Chapter 4

Variation in life-cycle between three rare and endangered floodplain violets in two regions: implications for population viability and conservation

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Abstract

We studied the demography of *Viola elatior*, *V. pumila*, and *V. stagnina*, three rare and endangered Central European floodplain species, to (i) analyse variation in life-cycles among congeners and between regions (Dyje-Morava floodplains, Czech Republic; Upper Rhine, Germany), (ii) to define sensitive stages in the life-cycles, and (iii) to identify possible threats for population viability and species conservation.

Matrix models were based on the fate of marked individuals from a total of 27 populations over two years. We analysed population growth rate (λ), stage distribution, net reproductive rate (R0), generation time, age at first reproduction, and elasticity and calculated a life table response experiment (LTRE).

Most populations were declining and λ did not differ between species or regions during the observed interval. Despite higher probabilities for survival and flowering in the Dyje populations, R0 was higher in the Rhine populations. Also other demographic traits showed consistent differences between regions and/or species. Complex life-cycles and large variation in λ precluded unequivocal identification of sensitive stages or vital rates for conservation. Variation between regions may be a consequence of differences in habitat quality.

Our results suggest that deterministic processes such as reduced management, succession, habitat destruction, and lack of disturbance through reduced or eliminated flooding present the strongest threat for the viability and persistence of populations of the three floodplain violets as compared with stochastic processes. However, the

persistent seed bank of the species may buffer populations against environmental variation and represents a reservoir for recovery after resumption of suitable land-use management.

Introduction

The growth and viability of populations ultimately depend on the vital rates: survival (stasis and regression), growth and reproduction (fecundity; cf. Caswell 2001). Environmental conditions (both abiotic conditions and biotic interactions) exert selective forces that may lead to the development of specific suites of traits in certain habitat types, along successional trajectories or along clines from the centre to the margin of the species range (Sagarin & Gaines 2002). Across species of different families, habitat-trait relationships go hand in hand with considerable differences among species, life-forms and functional groups in their life-cycles (Silvertown *et al.* 1993; Grime 2002) but to date there are only a few comparative studies that explicitly focussed on life-cycle differences among congeneric species (Newell *et al.* 1981; Solbrig *et al.* 1980, 1988; Svensson *et al.* 1993; Tolvanen *et al.* 2001).

However, in light of biodiversity loss and species conservation there is urgent need for detailed knowledge on variation in life-cycle among closely related species and within both common and rare species (e.g., Oostermeijer *et al.* 1996), because human domination of the earth's ecosystems and of biogeochemical processes increased the rates of species extinctions (Vitousek *et al.* 1997). Land-use changes are expected to exert the largest effects on biodiversity (Chapin *et al.* 1997; Vitousek *et al.* 1997; Sala *et al.* 2000). While land-use changes at the global scale denote the total conversion of ecosystems, e.g. from tropical forest to grassland or from grassland to crop fields, there may also be profound changes in ecosystem structure, function and composition as a consequence of intensification or de-intensification of land-use, especially within the cultural landscape of Europe (Korneck *et al.* 1998; Eriksson *et al.* 2002; Jacquemyn *et al.* 2003). Intensification of land-use will have direct negative effects through increased disturbance, fertilisation and the application of biocides and lead to species and habitat loss and a homogenisation of landscapes (Korneck *et al.* 1998; Brys *et al.* 2005). The abandonment of land-use and the following unguided succession processes will displace species of semi-natural habitats adapted to regular management (Jacquemyn *et al.* 2003). Due to the increased fragmentation of habitats, many (also formerly widespread) species have

become rare and restricted to small and/or isolated populations (e.g., Endels *et al.* 2002; Eriksson *et al.* 2002; Honnay *et al.* 2005). In Germany 28.4% of the total flora of flowering plants and ferns of 3319 species is red-listed (Korneck *et al.* 1996, 1998).

Rarity is closely related to the risk of extinction because species with narrow niche breadth are most likely to suffer more from variation or directional changes in external or internal conditions than widespread and common, generalist species (Gaston & Kunin, 1997). Within a species, the proportion of occupied sites and average population densities decline from the centre to the margin of its range (e.g., Lawton 1993; but see Sagarin & Gaines 2002 for a critical review). Therefore, peripheral plant populations are often more isolated (Lawton 1993; Lesica & Allendorf 1995) and contain fewer individuals than core populations (Durka 1999; Lammi *et al.* 1999; but see Kluth & Bruelheide 2005).

Since the future fate of organisms varies with age, stage or size, the demographic approach to conservation, i.e. population biological analyses of various aspects of the life-cycle as the fundamental unit for the description of organisms (Caswell 2001), promises to supply the necessary information for the conservation of rare and endangered species (e.g., Schemske *et al.* 1994; Menges 2000; Caswell 2001; Morris & Doak 2002; Nicolè *et al.* 2005).

The study species, *Viola elatior* Fr., *V. pumila* Chaix and *V. stagnina* Kit. ex Schult. (syn. *V. persicifolia* auct., non Schreber) are among the most endangered species across Europe deserving special conservation efforts (Schnittler & Günther 1999) and red-listed in many European countries (e.g., Korneck *et al.* 1996). Under the assumption of ecological niche stability, the species, which are today restricted mainly to the valleys of large lowland rivers with regional continental climate (Burkart 2001), were most probably more widely distributed and more frequent at the end of the last glaciation when climatic conditions in Central Europe were more continental. An analysis of grid maps suggests that the species have undergone a severe decline by about 50% during the last decades (Eckstein *et al.* 2006a), which caused strong fragmentation and isolation of populations (Eckstein *et al.* 2004; Eckstein *et al.* 2006 a, b). As such they belong to the 'new rares', which are probably more vulnerable to isolation and range contractions than 'naturally' rare plants (Huenneke 1991).

The aims of the present study were to (i) analyse and quantify differences in life-cycles among the endangered congeneric species and between regions with different position

to the species ranges, (ii) try to identify sensitive stages in the life-cycles based on the demographic approach, and (iii) summarise published evidence for stochastic and deterministic effects on population viability to evaluate possible threats to species conservation.

Material and Methods

Study species and regions

The study species and their habitats have been described in detail in Hölzel (2003), Eckstein *et al.* (2004), Eckstein and Otte (2005), and Eckstein *et al.* (2006 a). All three species are perennial iteroparous hemicryptophytes with a long-term persistent seed bank (Hölzel & Otte 2004). In Central Europe they occur mainly in the corridors of large lowland rivers. All three species show a Western Eurasian distribution (Hultén & Fries 1986), with Viola stagnina being relatively frequent also in the hemiboreal vegetation zone in Scandinavia.

The study was carried out in the Upper Rhine valley (Germany; in the following referred to as 'Rhine') and in the floodplains of the rivers Dyje and Morava in southern Moravia (Czech Republic; in the following 'Dyje'). The German populations are at the western margin of species ranges, while the Czech populations are close to the core distribution ranges (Hultén & Fries 1986).

Study design

The study was based on observations on the fate of all individual plants that occurred in marked permanent plots of 0.25 m2 (50 × 50 cm). Within each region, we selected three (*V. stagnina*), five (*V. pumila*) and five (*V. elatior*, Dyje) to six (*V. elatior*, Rhine) populations, which represented the characteristic vegetation types: the populations of *V. pumila* and *V. stagnina* were situated on extensively used sub-continental meadows mown once a year; the populations of *V. elatior* were situated in ecotonal habitats, such as grassland fringes and openings or clearings of alluvial forests adjacent to floodplain meadows. Depending on population size and extension, three to eight permanent plots were established in 2001 along a line transect across each population. Plots were selected to represent the variation in shoot density present across populations. In total, we investigated 1898 individuals in 122 plots from 27 populations. This study design, which seeks to attain a large (i.e. representative) spatial sample of populations, together with

logistic constraints and stochastic events (activities of wild boar), precluded excessive temporal replication. Therefore, the data only represent a single life-cycle transition. Since the sampling period was not extreme with respect to climate, we assume that it more or less represents an average year. However, as the temporal variation is unknown, the data should be interpreted with caution. Using largely the same data set, an analysis of the effects of management and environmental variation on density and frequency of life-cycle stages, i.e. the population stage structure, was presented by Eckstein *et al.* (2004). In contrast, the present paper develops a life-cycle of the three species and presents a detailed comparative analysis of the fates of individually marked plants based on the intrinsic population parameters obtained by matrix population models (cf. Caswell 2001).

Life-cycle stages

Seeds germinate in spring, and seedlings develop a few leaves by the end of summer. During May to early June generative plants may bear one to several open-pollinated (chasmogamous) flowers, which are potentially cross-pollinated and mature into capsules from early June to early July. Later in the season, cleistogamous flowers, which remain closed and are obligatorily self-pollinated, may be formed by the same individual (Eckstein & Otte 2005). The life-cycle stages used in the present paper were based on the presence of flowers and the number of shoots (cf. Eckstein *et al.* 2004):

(i) seedlings (s), defined by the presence of cotyledons,

(ii) small vegetative (v1), non-flowering plants, with one shoot,

(iii) large vegetative (v2), non-flowering plants with two or more shoots,

(iv) small generative (g1), flowering plants with one or two shoots,

(v) large generative (g2), flowering plants with three or more shoots.

The life-cycle stages as defined above differed markedly in their fates, i.e. their probabilities for survival, growth and reproduction (see below).

Matrix analyses

In each permanent plot all individual plants were recorded with their coordinates. With the density of shoots encountered in the field, we were able to assign each shoot to a particular single- or multiple-shoot plant. This approach was verified by careful

excavations of a few multiple-shoot plants outside our plots. Plots were re-visited in 2002, and the fate of all individuals (birth, death, survival in the same stage, regression into a lower stage, growth) was recorded. Using these data we prepared a 5 × 5 Lefkovitch matrix based on life-cycle stages (Caswell 2001; Appendix 1) for each population. Matrix elements (a_{ij}) represent probabilities for the transition between stage j in 2001 to stage i in 2002, except for the transitions a_{14} and a_{15} in the first row (Appendix 1), which express the average number of seedlings in 2002 produced per small or large flowering plant in 2001, respectively ('anonymous reproduction'; Caswell 2001). Anonymous reproduction also contains the dynamics of seeds in the seed bank. Since data on the number of cleistogamous capsules, which appear later in the year, are lacking, we assumed the same fertility in small and large generative plants. Consequently, a14 = a15 for the mean matrices, except if one of the stages was missing in one of the populations. To improve the generality of our results, all further analyses were based on a mean matrix per species × region combination that contained the mean values for each matrix entry of 3-6 single populations (Appendix 1).

To describe and compare the species' population dynamics we calculated the observed population growth rate (λ_O) and the bootstrapped estimate for λ (λ_B) together with accelerated 95% confidence intervals (Dixon 1993; McPeek & Kalisz 1993; Manly 2001). For the bootstrapping procedure we assumed a binomial distribution based on the observed numbers and probabilities of each stage class for matrix entries that represented transition probabilities. For fertilities we resampled (with replacement) new means for a_{14} and a_{15} based on our empirical estimates from the single populations (n=3-6) for each species-region combination. We restricted our bootstrap sample to matrices where no column sum of transition probabilities was larger than 1, since, by definition, transition probabilities for each stage class including death can only amount to 1. We did not apply a restriction with respect to the minimal column sums allowed, since any threshold would be arbitrary. However, column sums below 0.3 (i.e. a mortality of more than 70%) hardly occurred during resampling.

From the matrix analysis we obtained the stable stage distribution (SSD). Differences between observed stage distribution (OSD) and SSD were calculated as

$$\text{Keyfitz's } \Delta = 0.5 * \sum \left| x_i - w_i \right|,$$

where x_i and w_i are the observed and the expected proportions of stage i, respectively, to see whether the mean populations were in tune with their environment (Caswell 2001).
After decomposing the population matrix into one matrix containing only transition probabilities and another containing only fecundity we estimated age-specific traits from our stage-specific model as described in Caswell (2001: 116ff). We calculated net reproductive rates (R0), which give the mean number of offspring by which a newborn individual will be replaced by the end of its life, generation time (T), and the age at first reproduction (AFR) for seedlings conditional on survival. For each mean matrix we calculated elasticity (de Kroon *et al.* 1986), which represents the response of population growth rate to proportional changes in life-cycle transitions, i.e. the slope of log λ to log a_{ij}. We chose elasticity instead of sensitivity (response of λ to absolute changes in transitions), since elasticity sums to unity across each matrix, which makes comparisons of elasticity among species easier. Additionally, life-cycle elasticities, summarised for vital rates representing growth, stasis plus regression and fecundity can be compared with the elasticities spectrum of other species from the literature (Silvertown *et al.* 1996).
To analyse possible life-cycle differences between the two true meadow species *V. pumila* and *V. stagnina* and the ecotonal *V. elatior* and between regions (Rhine, Dyje), we calculated a two-way life table response experiment (LTRE; Caswell 1996, 2001). LTRE is a retrospective approach which decomposes the effects of species, region and their interaction on the observed difference in λ into contributions from different life-cycle transitions or vital rates (Caswell 1996). To this end, the differences in each matrix element (Δa_{ij}) between *V. elatior*, *V. pumila* and *V. stagnina* (mean matrices across regions), respectively, and an overall mean matrix (the mean matrix across species and regions served as 'reference matrix') are calculated. Then the sensitivity of λ to changes in each matrix element (sa_{ij}) was determined at a matrix 'half-way' between the two matrices compared (Caswell 1996, 2001). Finally, the contribution of each matrix element (ca_{ij}) was calculated as

$$ca_{ij} = \Delta a_{ij} * sa_{ij}.$$

Similarly the effects of region were calculated by comparison of regional mean matrices (across species) and the overall mean matrix. The models for main effects and their interaction were parameterized as given in Caswell (2001: eq.10.5-10.11). All contributions representing survival (P, stasis plus regression), growth (G) or fecundity (F) were summarised for each life-cycle stage. Positive (negative) contributions of certain

life-cycle transitions indicate that these transitions have a higher (lower) absolute value and/or a higher (lower) importance in certain species or regions than in the overall mean matrix. Matrix analyses were done using the program 'Poptools', version 2.6.7 (Hood 2005).

Log-linear analysis

For each species separately, we tested for the effects of region and life-cycle stage on the fate of individuals by using log-linear analysis (Caswell 2001; Quinn & Keough 2002). To meet assumptions of log-linear models of no more than 20% of categories having expected frequencies less than about five (Quinn & Keough 2002), we had to pool small and large generative plants into one stage class. Fates were defined as follows: (i) death, (ii) survival (i.e. stasis plus regression) and (iii) growth into a higher stage class. Log-linear analyses were done using Statistica (version 6.0, StatSoft Inc., Tulsa, USA).

Results

Matrix analysis

Observed population growth rates (λ_O) for the 2001-2002 transition were <1 for all species-region combinations, except for the Rhine populations of *V. stagnina* (Table 4.1). Bootstrap estimates of λ (λ_B) were slightly lower than λ_O, and their 95% confidence intervals were very close to but did not include a value of λ_B=1, again with the exception of *V. stagnina* from the Rhine. Values for λ_B did not differ significantly among species within the same region or between regions (Table 4.1). Across species the proportion of flowering plants was slightly higher in populations of the Dyje floodplains than at the Rhine (data not shown). Flowering was not synchronised among species since the proportion of flowering plants decreased between years in *V. elatior* and *V. pumila* but increased in *V. stagnina*. Despite similar or higher proportions of flowering plants in the Dyje populations, net reproductive rates (R0) of *V. pumila* and *V. stagnina* were lower here than in the Rhine populations (Table 4.1). This was mainly due to large differences in the numbers of expected offspring of *V. stagnina* between regions. Seedlings of the species showed an age at first reproduction of about four years (Table 4.1), i.e. an average seedling produced seeds three years after the year of its germination. Seedlings of *V. stagnina* from the Dyje populations reproduced at an age of five years,

Table 4.1 Observed population growth rate (λ_O), bootstrap estimate of λ (λ_B) with lower and upper 95% bootstrap confidence intervals CI(l) and CI(u), respectively, net reproductive rate (R0), generation time (T), and age at first reproduction (AFR) of *Viola elatior*, *V. pumila* and *V. stagnina* in their main Central European regions of occurrence (Dyje, Czech Republic; Rhine, Germany).

Region	Species	λ_O	λ_B	CI(l)	CI(u)	R0	T	AFR
Dyje	*Viola elatior*	0.844	0.788	0.493	0.946	0.340	6.38	4.02
	Viola pumila	0.761	0.704	0.400	0.870	0.140	7.19	4.17
	Viola stagnina	0.888	0.827	0.663	0.912	0.061	23.47	4.85
Rhine	*Viola elatior*	0.810	0.803	0.625	0.898	0.273	6.17	4.17
	Viola pumila	0.881	0.863	0.652	0.989	0.366	7.94	4.60
	Viola stagnina	1.121	1.076	0.750	1.276	1.870	5.50	3.28

while they reached maturity with three years, i.e. slightly earlier than *V. elatior* and *V. pumila*, in the Rhine floodplains.

When the observed stage distributions were compared between species pairs, *V. elatior* and *V. pumila* showed the highest degree of similarity, while OSD of *V. stagnina* differed strongly from the other two species. Observed stage structures of species pairs were more similar in populations of the Dyje river than at the Rhine (data not shown). *Viola pumila* showed the smallest differences between expected and observed stage distributions (Table 4.2) which was indicated by a low Keyfitz's Δ. In *V. elatior*, the largest positive deviations from the expected stage proportions (i.e. more observed than expected from SSD) were observed for seedlings, the largest negative deviations for large vegetative (Rhine) and small flowering plants (Dyje). In *V. stagnina* deviations from the SSD were almost exactly opposite in the two regions, and the largest contribution to the observed dissimilarity was through small vegetative and large flowering plants.

Elasticities

Across species elasticities for survival were between 0.25 and 0.75, those for growth between 0.25 and 0.50, and those for fecundity between 0 and 0.25 (Fig. 4.1). Elasticities for the Rhine and Dyje populations were very similar in Viola elatior, whereas variation between regions was larger in *V. pumila* and, especially, in *V. stagnina*. The elasticity of λ for fecundity was close to zero in the Dyje populations of *V. stagnina*, whereas the Rhine populations of this species had the highest elasticity for fecundity of all species-region combinations.

Table 4.2 Observed (OSD) and expected stable stage distribution (SSD) of *Viola elatior*, *V. pumila* and *V. stagnina* from populations at the Dyje and the Rhine. Keyfitz's Δ measures the distance between SSD and OSD for each species-region combination. Stages: s, seedling; v1, small vegetative; v2, large vegetative; g1, small generative; g2, large generative.

Viola elatior, Dyje	OSD	SSD	Difference	Keyfitz's Δ
s	0.308	0.224	0.084	0.134
v1	0.277	0.323	-0.046	
v2	0.246	0.196	0.051	
g1	0.108	0.194	-0.086	
g2	0.062	0.064	-0.002	
Viola elatior, Rhine				
s	0.354	0.262	0.092	0.126
v1	0.382	0.412	-0.030	
v2	0.135	0.220	-0.085	
g1	0.055	0.066	-0.011	
g2	0.075	0.040	0.034	
Viola pumila, Dyje				
s	0.256	0.239	0.017	0.036
v1	0.376	0.369	0.007	
v2	0.214	0.228	-0.015	
g1	0.068	0.057	0.012	
g2	0.085	0.107	-0.022	
Viola pumila, Rhine				
s	0.217	0.287	-0.070	0.077
v1	0.346	0.298	0.047	
v2	0.275	0.250	0.025	
g1	0.075	0.070	0.005	
g2	0.088	0.095	-0.007	
Viola stagnina, Dyje				
s	0.007	0.022	-0.014	0.183
v1	0.360	0.197	0.163	
v2	0.272	0.321	-0.049	
g1	0.162	0.142	0.020	
g2	0.199	0.319	0.120	
Viola stagnina, Rhine				
s	0.611	0.559	0.051	0.143
v1	0.095	0.231	-0.137	
v2	0.053	0.054	-0.001	
g1	0.084	0.090	-0.005	
g2	0.158	0.066	0.092	

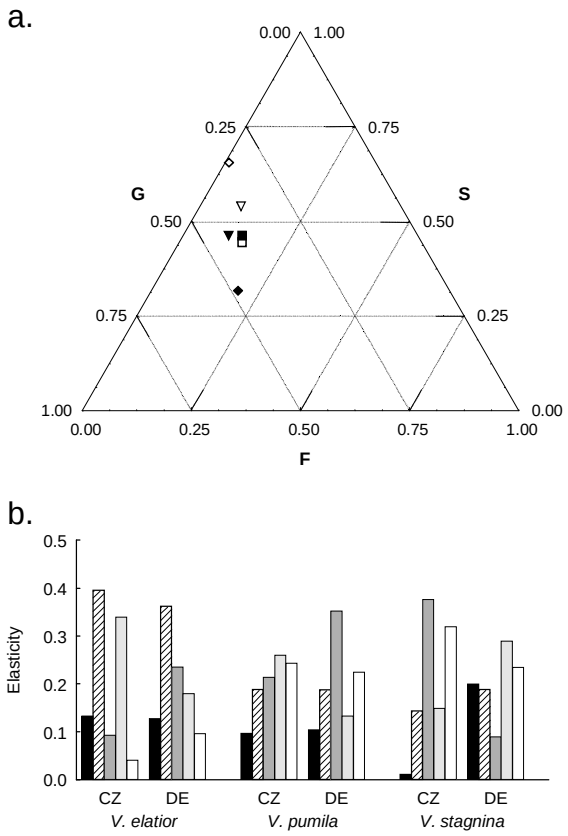

Figure 4.1 Elasticity of population growth rates to survival (S), growth (G), and fecundity (F) for *Viola elatior* (squares), *V. pumila* (triangles) and *V. stagnina* (diamonds) from the Rhine (filled symbols) and Dyje (open symbols) floodplains (a.) and stage-specific elasticities in the Dyje (CZ) and Rhine (DE) populations (b.). Stages: seedling (black); small vegetative (hatched); large vegetative (dark grey); small generative (light grey); large generative (white).

Stage-specific elasticities were relatively similar in both regions in *V. elatior* and *V. pumila* (Fig. 4.1). However, while the fate of small vegetative plants had the largest effect on λ in the former, small and large adults had a roughly equal effect in the latter. In contrast, there were large differences in the contribution of different stages to λ between regions in *V. stagnina*. In the Dyje populations the fate of large plants was most important with almost no effect of variation in seedling fates, whereas along the Rhine flowering plants and seedlings had the largest effects.

LTRE

The predicted population growth rates (λ) based on our LTRE were within 2.5% of the observed values for λ, which showed that our linear models were very accurate (Caswell 1996, 2001). Absolute differences in life-cycle transitions between species-region combinations and the overall mean matrix were about ±0.3, whereas differences in fecundity were even >0.3 (not shown). In comparison with the overall mean matrix

(λ=0.9085), populations across regions of Viola elatior (λ=0.8602) and *V. pumila* (λ=0.8261) had lower growth rates. These differences were accounted for by negative contributions of growth and fecundity (Fig. 4.2). Higher λ of *V. stagnina* across regions (λ=1.0565) was owing to positive contributions of growth and fecundity (Fig. 4.2).

Across species, the Dyje and the Rhine populations had population growth rates of 0.8464 and 0.9294, respectively. Survival did not contribute to the difference in population growth between the overall mean matrix and the regional matrices across species. Negative contributions of growth transitions were outweighed by positive contributions of fecundity in the Rhine populations, whereas the pattern was exactly opposite in the Dyje populations (Fig. 4.2). There was a species-region interaction, which was indicated by a much closer match between observed and predicted λ when the interactions term was considered than when only accounting for the additive effects of species and region (not shown). Especially, the performance of the Dyje populations of *V. stagnina* and of the Rhine populations of *V. elatior* was overestimated when considering only additive effects.

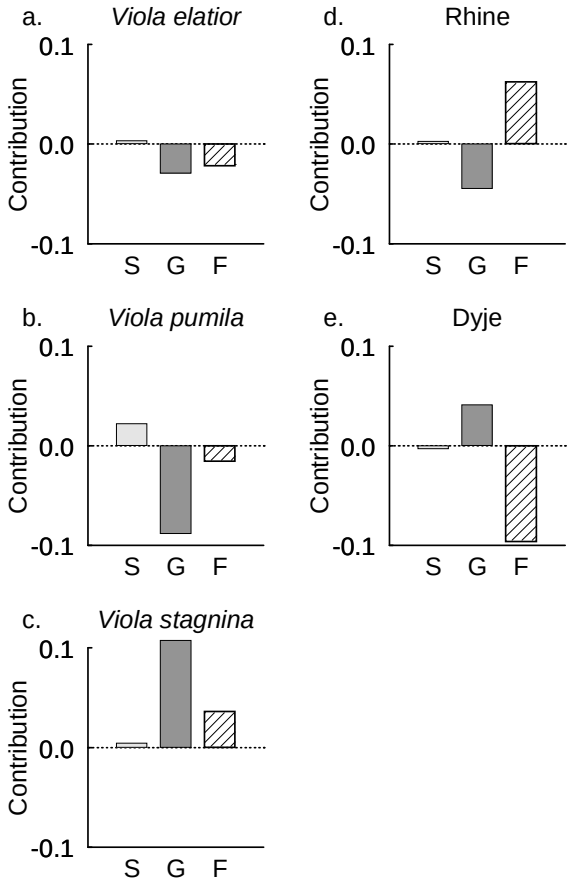

Figure 4.2 Results of a two factor life table response experiment (LTRE). Given are the contributions of survival (S_i, stasis plus regression, light grey bars), growth (G_i, dark grey bars) and fecundity (F_i, hatched bars) to the differences in population growth rate (Lambda) between species (a. *Viola elatior*, b. *V. pumila*, c. *V. stagnina*) and regions (d. Rhine, e. Dyje) as compared to an overall mean matrix across species and regions. Stages *i*: 1, seedling; 2, small vegetative; 3, large vegetative; 4, small flowering; 5, large flowering plant.

In summary the high performance of the Rhine populations of *V. stagnina* had a strong influence on both the mean λ of this species across regions and on the mean λ of Rhine populations across species. Especially fecundity contributed to the difference in λ between the Dyje and Rhine populations of *V. stagnina*.

Log-linear analysis

Results from the log-linear analyses showed that there were significant differences in fate among stages, even when the effect of regions already was accounted for in the model (significant L and L(|R) effects, Table 4.3). The probability of survival from 2001 to 2002 varied between 0.2 and 1.0 among stages and regions (Fig. 4.3). It generally increased with stage class and showed little variation among species. Seedlings experienced a much higher mortality (45-80%) than adult stages (<40%). Low mortalities of adult stages and low numbers of observed individuals in some small Dyje populations lead to survival probabilities of 1.0 for flowering plants of *V. elatior* and *V. pumila* which obviously are an over-estimation in the long run. Similarly, probability of flowering during the next year increased with stage class (Fig. 4.3).

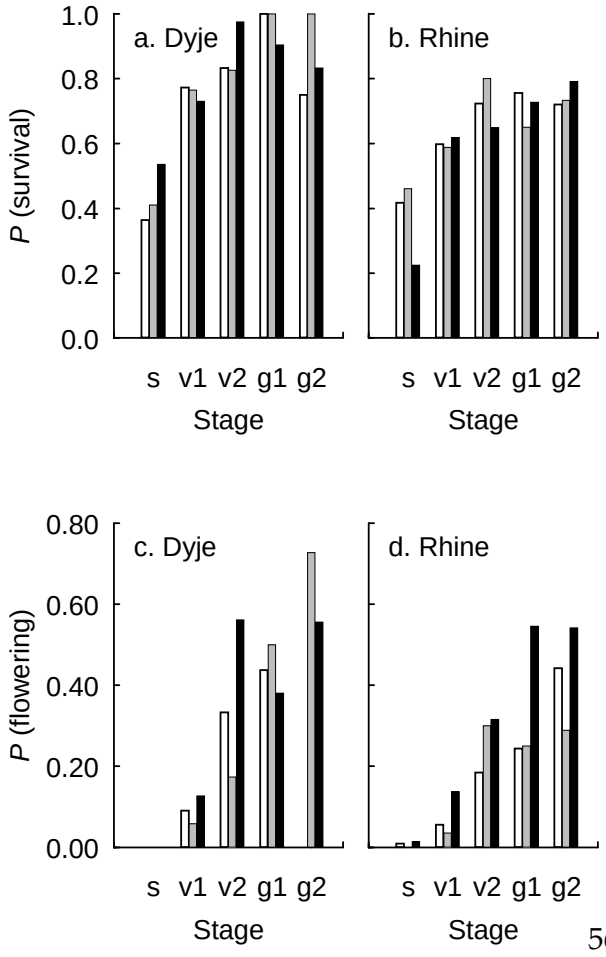

Figure 4.3 Probability of survival (a., b.) and flowering (c., d.) of different life-cycle stages (s, seedling; v1, small vegetative; v2, large vegetative; g1, small generative; g2, large generative) of *Viola elatior* (white bars), *V. pumila* (grey bars) and *V. stagnina* (black bars) from the Rhine (b., d.) and Dyje (a., c.) floodplains.

It increased from 0-1% in seedlings and 4-13% in small vegetative plants to 20-70% in the other stages. In Rhine populations, *V. stagnina* showed the highest flowering probabilities. The probability of repeated flowering was as high as or higher than the probability for vegetative plants to flower during the next year in most cases (Fig. 4.3). Zero probability of repeated flowering of large plants of *V. elatior* was most probably a sampling effect due to a small sample size (n=4). Differences between regions in the fate of individual plants were not or marginally significant in *V. pumila* and *V. elatior* (Table 4.3), while in *V. stagnina* population turn-over was significantly higher in the Rhine populations than in the Dyje populations, even when the effect of stage was accounted for.

Table 4.3 Results of a log-linear analysis on the effects of *region* (R; Dyje, Rhine) and *life cycle stage* (L; seedling, small vegetative, large vegetative, reproductive) on the fate (death, regression/stasis, growth) of the individuals of *Viola elatior*, *V. pumila* and *V. stagnina*. The columns 'delta Chi²' and 'delta DF' give the differences in Chi² and degrees of freedom between a null-model and one that includes the effect of a certain factor on fate. Abbreviations: L(|R) and R(|L) denotes the effect of factor L (R) when the effects of factor R (L) on fate is already included in the null-model, and R * L denotes the interaction between *region* and *life-cycle stage*.

Viola elatior	**Factor**	**delta Chi²**	**delta DF**	***p***
	R	5.87	2	0.0531
	L	161.26	6	<0.0001
	R(|L)	4.59	2	0.1008
	L(|R)	159.98	6	<0.0001
	R * L	6.27	6	0.3936
Viola pumila	**Factor**	**delta Chi²**	**delta DF**	***p***
	R	0.45	2	0.7985
	L	261.41	6	<0.0001
	R(|L)	6.85	2	0.0325
	L(|R)	267.81	6	<0.0001
	R * L	8.31	6	0.2163
Viola stagnina	**Factor**	**delta Chi²**	**delta DF**	***p***
	R	117.85	2	<0.0001
	L	398.94	6	<0.0001
	R(|L)	25.89	2	<0.0001
	L(|R)	304.70	6	<0.0001
	R * L	10.58	6	0.1023

Discussion

In the present study the fate of individuals in permanent plots was followed over one transition. This temporal non-replication of matrices will preclude the possibility for stochastic modelling (i.e. modelling population growth through randomly selecting different matrices of different years), because temporal variation should not be substituted by spatial variation in stochastic models (Jongejans & de Kroon 2005). On the other hand, the present study is based on a large data set, comparing the life-cycle of three congeneric species. It relies on observations from a total of 27 populations (6-11 populations per species) from two different regions with respect to the species range.

Population growth rates of all but one species-region combination were <1 during the study interval. Confidence intervals around λ based on matrix entries averaged across populations showed that species and regions did not differ significantly in growth rates. Since λ is influenced by climatic conditions this indicates that conditions did not differ substantially (at least with respect to their effect on λ) between regions. A long-term λ of around 1 can be expected in perennials as long as the populations do not go extinct (e.g., Solbrig *et al.* 1980; Newell *et al.* 1981; Silvertown *et al.* 1993; Svensson *et al.* 1993; Oostermeijer *et al.* 1996; Nicolè *et al.* 2005).

Life-cycle analysis revealed considerable differences among species and between regions in a number of characteristics. Based on the pooled number of transitions per species-region combination, probabilities for survival were higher for the Dyje populations than for the Rhine populations (Fig. 4.3). Similarly, the probability of flowering was as high or higher for vegetative plants, and the probability of repeated flowering was higher in *V. pumila* in the Dyje populations than in the Rhine populations. The Dyje populations of *V. elatior* and *V. stagnina* contained a higher proportion of flowering plants than did the Rhine populations. Despite this, the net reproductive rate (R0) was higher in the Rhine populations of *V. pumila* and *V. stagnina* and only slightly lower than in the Dyje populations in *V. elatior*. This, together with higher seedling densities of *V. elatior* and *V. stagnina* in populations at the Rhine (Eckstein *et al.* 2004), indicated that habitat quality may be worse in the Dyje floodplains than along the Rhine. We suggest that two aspects may be important: (1) In the Dyje populations, capsules of the study species were infested by larvae of *Orbitis cyaneus* (L.), a beetle (Coleoptera) of the Curculionidae family, while this phenomenon has not been observed in the Rhine populations. Larvae live inside the capsules, feeding on developing seeds.

Consequently, infested capsules develop only a few or no mature seeds. In marked individual plants outside the permanent plots, infestation rates of capsules varied between 9.7 and 66.7% across capsule types (chasmogamous, cleistogamous), species and years (2002, 2003; Lučeničová, unpubl.). These data suggest that insects can substantially reduce seed production. (2) Another important difference between study regions is the frequency of management, mostly mowing or mulching (Eckstein *et al.* 2004). The Rhine populations are situated exclusively in nature conservation areas or are managed under conservation contracts, whereas the Dyje populations are found mainly in irregularly managed meadows. Many species from semi-natural habitats respond negatively to habitat deterioration, abandonment and fragmentation (e.g., Jacquemyn *et al.* 2003; Brys *et al.* 2005; Lindborg *et al.* 2005), whereas regular management reduces the asymmetry in competition for light and slows down the accumulation of litter and encroachment by bryophytes thereby improving population viability and individual performance (Lepš 1999; Lennartsson & Oostermeijer 2001; Endels *et al.* 2002; Brys *et al.* 2005). Seedling density was negatively correlated with bryophyte cover, and regular management had positive effects on seedling density and population stage structure in *V. elatior* and *V. stagnina* (Eckstein *et al.* 2004). These findings are corroborated by significant or marginally significant effects of region on the fates of individuals in *V. stagnina* and *V. elatior* in the present study.

Though germination of *V. elatior* and *V. pumila* increased with increasing litter cover especially under dry conditions (Eckstein & Donath 2005; no data for *V. stagnina*), the release of seeds onto a dense carpet of litter, which impedes seed-soil contact, generally has negative effects on seed survival and germination (Facelli & Pickett 1991 and references therein). The above aspects of habitat quality are not exclusive but may act in concert to reduce net reproductive rate of the Dyje populations, leading to a regressive population stage structure and increased extinction risk in the long run (Oostermeijer *et al.* 1994).

A number of plant species from semi-natural habitats have a dynamic population stage structure, i.e. a high proportion of seedlings and juveniles, in response to high habitat quality (Jensen & Meyer 2001; Colling *et al.* 2002), and especially regular land-use such as grazing or mowing (Oostermeijer *et al.* 1994; Bühler & Schmid 2001; Lennartsson & Oostermeijer 2001; Bissels *et al.* 2004), while populations under sub-optimal conditions show a regressive stage structure, i.e. a high proportion of adult plants and no or only a

few seedlings (Endels *et al.* 2002). Especially increased competition for light has negative effects on growth, flowering, seed production and seedling recruitment of other violet species from semi-natural habitats (Jensen & Meyer 2001; Moora *et al.* 2003). *Viola stagnina* showed the largest variation in life-cycle between regions, which may indicate that this species would profit most from regular management (Pullin & Woodell 1987) and soil disturbance through flooding and animal activity, which may enhance germination of dormant seeds from the soil seed bank (Croft & Preston 1996).

Elasticity analysis revealed that despite some differences between regions, small vegetative and small and large generative plants contributed most to λ in *V. elatior*, whereas all four adult stages contributed roughly similar to λ in *V. pumila*. In contrast, the contribution of different stages and vital rates varied strongly between regions in *V. stagnina*. In growing populations the contributions of growth and fecundity increased, which has also been confirmed for *Gentiana pneumonanthe* (Oostermeijer *et al.* 1996), and for *Cirsium vulgare* and *Pedicularis furbishiae* (Silvertown *et al.* 1996). Elasticities of vital rates varied between years and populations in *Agrimonia eupatoria* and *Geum rivale* (Kiviniemi 2002) and *Primula vulgaris* (Valverde & Silvertown 1998), whereas they were relatively constant in three species of *Pinguicula* (Svensson *et al.* 1993). The fact that matrix transitions are not independent of each other and that elasticity varies with population growth rate (Oostermeijer *et al.* 1996; Silvertown *et al.* 1996; Caswell 2001) makes it difficult to identify unique stages or vital rates as sensitive phases or processes for conservation or management. Additionally, since elasticity is calculated as sensitivity multiplied by the quotient of the transition value (a_{ij}) and λ (de Kroon *et al.* 1986; Caswell 2001) common transitions will often also have a high elasticity, whereas rare transitions may be better targets for conservation or recovery plans. For example, fecundity in *V. stagnina* from the Dyje floodplains was already very low owing to low habitat quality (missing or infrequent management), which, in turn, led to elasticities close to zero, indicating that this transition (and the seedling stage) did not contribute significantly to λ. However, the populations will probably not resume positive growth unless reproduction and germination are enhanced through changes in management or disturbances improving conditions for the germination of seeds from the seed rain or the persistent seed bank.

Studies on the pollination biology and population genetic structure of the study species showed that owing to their chasmogamous-cleistogamous mating system, the three

floodplain violets are not pollen limited, and there are no signs of inbreeding depression (Eckstein & Otte 2005). Additionally, despite strong genetic divergence between populations of all species and the loss of some genetic markers in marginal populations of *V. stagnina*, there was no statistically significant relationship between genetic diversity and population size (Eckstein, unpubl.) or isolation and no equilibrium between genetic drift and gene flow (Eckstein *et al.* 2006 b). Therefore, demographic stochasticity (this study), environmental stochasticity (Eckstein *et al.* 2004), pollen limitation and inbreeding depression (Eckstein & Otte 2005) as well as genetic stochasticity (Eckstein *et al.* 2006 b) are unlikely to present a major threat to population viability and persistence as compared to deterministic processes.

However, as in many other species the presence of a persistent seed bank probably is an efficient buffer against environmental variation and successional change (e.g., Solbrig *et al.* 1988; McCue & Holtsford 1998; Cabin & Marshall 2000; Adams *et al.* 2005). Consequently, the floodplain violets may show strong extension and contraction of population size along successional trajectories, starting with a burst of germination after a major disturbance. With the density of adults increasing, germination and seedling survival decrease and population size slowly decreases with time until populations only persist as seeds in the soil seed bank. This has been described as remnant regional population dynamics (Eriksson 1996).

Conclusions and management recommendations

The results of the present demographic study together with information from other published papers on different aspects of the ecology of the study species suggest that deterministic processes present the strongest threat for the viability and persistence of populations of the three floodplain violets (Korneck *et al.* 1998; Eckstein *et al.* 2006 a). These processes comprise reduced management of the non-intensively used sub-continental floodplain meadows, the succession from open forest fringes and paths to closed mature alluvial forests (concerning especially *V. elatior*), habitat destruction, and reduced disturbance through flooding. These processes decrease habitat quality through litter accumulation, bryophyte encroachment and colonization by shrubs and trees. Consequently, seed germination is reduced and population size decreases. The persistent seed bank of the species presents a buffer against environmental variation

and a reservoir for the recovery of populations after resumption of suitable management.

Together with information from other studies on the floodplain violets (Hölzel 2003; Eckstein *et al.* 2004; Eckstein & Otte 2005; Hölzel *et al.* 2006; Eckstein *et al.* 2006 a, b), results of the current study suggest that the most suitable management of the meadow species *V. pumila* and *V. stagnina* consists of regular mowing. Depending on the weather and on local conditions, meadows with these species should be mown once (May/June) or twice (May/June; July/August) per year. Mowing should not be done too late to efficiently suppress competitors and to allow regrowth and seed development of the violets. Management as meadow pastures, e.g. aftermath grazing with sheep or non-intensive pasture use appears to be also suitable. However, short-duration grazing seems to be preferable to permanent pastures. Regular mowing or grazing will keep the stands open and reduced competition will improve recruitment and increase the proportion of seedlings in these two species (Eckstein *et al.* 2004; this study). Unlike some other species-rich grasslands with low productivity such as Mesobromion communities, extensively managed temperate floodplain meadows of the alliances Arrhenaterion and Cnidion at the Upper Rhine are characterised by annual yields of between 350 and 470 g m^{-2} (Donath *et al.* 2004). Regarding fodder quality, hay from these stands is well suited as basic ration for feeding systems with cattle and horses (Donath *et al.* 2004).

Viola elatior has a lower mowing compatibility and thus avoids early or regularly mown meadows (Hölzel 2003). However, also this species will profit from regular meadow management, which includes the occasional mowing of the ecotonal fringe between hay meadows and alluvial forests, and thus prevents the expansion of shrubs and trees. Owing to a more suitable light regime and rotation times of 30-40 years traditional simple coppice or coppice-with-standards management appears to be more suitable than the common timber forest use with long rotation times (120 years).

As the current study shows, regular management is crucial for the violet species. Suitable land use on the other hand will enable the conservation of viable population of these endangered plant species even at the margin of the distribution range.

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Appendix 1: Mean population matrices for transitions from 2001 to 2002 of *Viola elatior*, *V. pumila* and *V. stagnina* in their main Central European regions of occurrence (Dyje, Czech Republic; Rhine, Germany). Numbers below and to the right of the stages give the number of individuals (n) observed in 2001 and 2002, respectively. Matrix elements (aij) represent probabilities for the transition between stage j in 2001 to stage i in 2002, except for the transitions a14 ('small generative to seedling') and a15 ('large generative to seedling') in the first row; numbers given there express the average number of seedlings in 2002 produced per small and large flowering plant in 2001 ('anonymous reproduction', Caswell 2001). n = number of populations.

Viola elatior	**2001**	seedling	small	large	small	large
Rhine (*n*=6)			vegetative	vegetative	generative	generative
2002	*n*	*103*	*179*	*65*	*41*	*43*
Seedling	*142*	*0*	*0*	*0*	*2.003*	*2.003*
small vegetative	*153*	*0.434*	*0.363*	*0.237*	*0.272*	*0.012*
large vegetative	*54*	*0*	*0.159*	*0.378*	*0.319*	*0.210*
small generative	*22*	*0.004*	*0.076*	*0.029*	*0.200*	*0.024*
large generative	*30*	*0*	*0.006*	*0.073*	*0.048*	*0.276*

Viola pumila	2001	seedling	small	large	small	large
Rhine (*n*=5)			vegetative	vegetative	generative	generative
2002	*n*	*126*	*85*	*80*	*40*	*45*
Seedling	*64*	*0*	*0*	*0*	*1.532*	*1.532*
small vegetative	*102*	*0.491*	*0.272*	*0.094*	*0.113*	*0.099*
large vegetative	*81*	*0.008*	*0.210*	*0.386*	*0.336*	*0.370*
small generative	*22*	*0*	*0.046*	*0.114*	*0.189*	*0.065*
large generative	*26*	*0*	*0*	*0.179*	*0.090*	*0.345*

Viola stagnina	2001	seedling	small	large	small	large
Rhine (*n*=3)			vegetative	vegetative	generative	generative
2002	*n*	*494*	*131*	*57*	*11*	*24*
seedling	*100*	*0*	*0*	*0*	*4.021*	*4.021*
small vegetative	*152*	*0.338*	*0.246*	*0.075*	*0.067*	*0.048*
large vegetative	*42*	*0.005*	*0.109*	*0.216*	*0.111*	*0.158*
small generative	*35*	*0.012*	*0.107*	*0.239*	*0.333*	*0.396*
large generative	*27*	*0.022*	*0.052*	*0.190*	*0.244*	*0.268*

Viola elatior	2001	seedling	small	large	small	large
Dyje (*n*=5)			vegetative	vegetative	generative	generative
2002	*n*	*11*	*22*	*6*	*16*	*4*
seedling	*20*	*0*	*0*	*0*	*0.689*	*0.589*
small vegetative	*18*	*0.400*	*0.481*	*0*	*0.117*	*0*
large vegetative	*16*	*0*	*0.079*	*0.150*	*0.383*	*0.333*
small generative	*7*	*0*	*0.267*	*0.200*	*0.283*	*0*
large generative	*4*	*0*	*0*	*0.050*	*0.217*	*0*

Viola pumila	2001	seedling	small	large	small	large
Dyje (*n*=5)			vegetative	vegetative	generative	generative
2002	*n*	*83*	*17*	*23*	*10*	*11*
seedling	*30*	*0*	*0*	*0*	*1.373*	*0.973*
small vegetative	*44*	*0.603*	*0.261*	*0.167*	*0.040*	*0.000*
large vegetative	*25*	*0*	*0.132*	*0.267*	*0.280*	*0.450*
small generative	*8*	*0*	*0.029*	*0.000*	*0.480*	*0.050*
large generative	*10*	*0*	*0*	*0.167*	*0.200*	*0.300*

Viola stagnina	2001	seedling	small	large	small	large
Dyje (*n*=3)			vegetative	vegetative	generative	generative
2002	*n*	*28*	*63*	*41*	*21*	*18*
seedling	*1*	*0*	*0*	*0*	*0.042*	*0.042*
small vegetative	*49*	*0.667*	*0.443*	*0.084*	*0.325*	*0*
large vegetative	*37*	*0*	*0.172*	*0.392*	*0.230*	*0.291*
small generative	*22*	*0*	*0.093*	*0.269*	*0*	*0.067*
large generative	*27*	*0*	*0.026*	*0.238*	*0.397*	*0.456*

Chapter 5

Effects of cleistogamy and pollen source on seed production and offspring performance in three endangered violets

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Abstract

In rare plants that often occur in small or isolated populations the probability of selfing between close relatives is increased as a consequence of demographic stochasticity. The mode of pollination (selfing, outcrossing) may have considerable effects on seed traits and offspring performance and hence potential viability. Since current efforts aiming at the restoration of floodplain grasslands through the transfer of plant material from species-rich source stands may lead to the establishment of initially small populations consisting of founders from different populations, the present paper experimentally investigated the effects of pollen source and floral types (i.e. chasmogamous (CH) and cleistogamous (CL) flowers) on seed traits and offspring performance in three highly endangered violet species (*Viola elatior*, *V. pumila*, *V. stagnina*) of these grasslands. We estimated inbreeding depression and tested the performance of selfed and outcrossed offspring in two microbial environments, i.e. in soil inoculated with (i) non-sterile substrate from the same species ('home'-conditions) and (ii) sterilised substrate.

Plants produced more CL capsules than CH flowers. Pollinator exclusion had only small effects on CH seed production. CL seeds had a significantly lower mass per seed than CH seeds. This may be related to constraints in allocation or environmental conditions. Seedling growth was reduced in plants grown under 'home'-conditions as compared to control soils. Under 'home'-conditions, relative fitness of selfed seedlings of *V. stagnina* was significantly higher than that of crossed progeny. Our results suggest that high genetic differentiation among populations as a consequence of isolation may result in

outbreeding depression, e.g., though biochemical or physiological incompatibilities between genes or the breaking of coadapted gene complexes. In *V. stagnina*, offspring fitness differed considerably between environments, but in general we found no indications for inbreeding depression in these rare species.

Zusammenfassung

Bei seltenen Arten, die oft in kleinen oder isolierten Populationen vorkommen, ist die Wahrscheinlichkeit von Selbstbestäubung durch verwandte Individuen als Folge demographischer Stochastizität erhöht. Die Art der Blütenbestäubung kann erhebliche Auswirkungen auf Samenmerkmale und die Fitness der Nachkommen und daher auf die Lebensfähigkeit von Populationen haben. Zur Zeit durchgeführte Maßnahmen zur Wiederherstellung von Stromtalwiesen durch die Übertragung von Pflanzenmaterial aus artenreichen Spenderbeständen können zur Entstehung von anfangs kleinen, aus Individuen verschiedener Spenderbeständen zusammengesetzten Populationen führen. Daher untersucht die vorliegende Arbeit die Effekte unterschiedlicher Pollenquellen und des Blütentyps (chasmogame (CH) und kleistogame (CL) Kapseln) auf Samenmerkmale und die Fitness der Nachkommen von drei stark gefährdeten Veilchenarten (*Viola elatior*, *V. pumila* und *V. stagnina*) dieser Habitate. Wir ermittelten den Grad der Inzuchtdepression und untersuchten das Keimlingswachstum von Nachkommen aus Selbst- und Fremdbestäubung unter Bedingungen, die sich in Hinblick auf die mikrobielle Umwelt unterschieden, d.h. in Boden, der mit (i) nicht-sterilem Substrat derselben Art (‚Heimat'-Bedingungen) und (ii) sterilisiertem Substrat beimpft war. Die Veilchen produzierten mehr CL Kapseln als CH Blüten. Der experimentelle Ausschluss von Bestäubern hatte nur geringe Auswirkungen auf die Produktion von CH Samen. CL Samen hatten im Mittel ein signifikant geringeres Gewicht als CH Samen. Dies kann mit unterschiedlicher Allokation von Ressourcen oder unterschiedlichen Umweltbedingungen bei der Samenbildung zusammenhängen. Das Keimlingswachstum war geringer unter ‚Heimat'-Bedingungen als unter Kontrollbedingungen. Unter ‚Heimat'-Bedingungen war bei *V. stagnina* die relative Fitness von Keimlingen aus Selbstbestäubung signifikant höher als die von fremdbestäubten Nachkommen. Unsere Ergebnisse legen nahe, dass ein hoher Grad genetischer Differenzierung als Folge der Isolation der Populationen dieser Art zu Kreuzungsdepression, z. B. durch biochemische oder physiologische Inkompatibilität

zwischen Genen oder den Bruch koadaptierter Genkomplexe, führen könnte. Die Fitness von Nachkommen von *V. stagnina* variierte stark zwischen den Bodenbehandlungen, aber generell fanden wir keine Hinweise auf Inzuchtdepression bei den untersuchten seltenen Veilchenarten.

Introduction

Many plant species that have been more widespread in the past, are today restricted to small and isolated populations as a result of land use changes, habitat destruction and fragmentation (e.g., Saunders, Hobbs & Margules 1991; Jensen & Schrautzer 1999; Kahmen & Poschlod 2000; Dudash & Fenster 2000; Lienert; Diemer & Schmid 2002). Small and isolated populations meet an increased risk of extinction through environmental stochasticity or catastrophes (Lande 1993). However, viability of these populations may also be reduced because of the increased chance of mating between relatives in small populations. Pollen may originate from anthers of the same flower (autogamous selfing), from a different flower on the same shoot or genet (geitonogamous selfing), or a shoot from another genet (xenogamy, crossing) (Richards 1997). Thus especially in small and/or isolated populations there is a large probability for self pollination (Barrett & Kohn 1991; Dudash & Fenster 2000).

Selfing may lead to inbreeding depression (e.g., Husband & Schemske 1996; Culley 2000; Dudash & Fenster 2000; Charlesworth 2003), i.e. the reduction in viability and fitness (Waller 1984; Mitchell-Olds & Waller 1985; Dudash 1990; Carr; Murphy & Eubanks 2003) in selfed as compared to outcrossed progeny, leading to increased abortion of ovules, and reduction in seed set, germination, offspring fitness and population genetic structure (Waller 1984; Dudash 1990; Fischer & Matthies 1997; Richards 1997; Berg & Redbo-Torstensson 1999; Culley 2000; Karrenberg & Jensen 2000; Lienert & Fischer 2002).

While most open self-compatible flowers are potentially cross or self-pollinated, there is an extreme case of floral reduction in cleistogamous (CL) flowers (Richards 1997), which are obligatorily selfed. These occur in at least 256 species from 56 angiosperm families (Lord 1981). In 'true' cleistogamous species (Plitmann 1995) both open, potentially cross-pollinated flowers (chasmogamous flowers, CH) and CL flowers may develop on the same individual. The CH-CL system provides a mixed-mating system with both potentially variable (CH) and relatively invariable (CL) offspring, that may represent a

'fail-safe' or 'bet-hedging' strategy, optimising reproductive output in fluctuating environments (Berg & Redbo-Torstensson 1998; but see Mattila & Salonen 1995). However, to our knowledge, there are only few studies that explicitly differentiated between pollination mode and floral types (e.g., Culley 2000), while in most cases CH flowers were simply assumed to be outcrossed. Furthermore, despite a wealth of information on inbreeding depression (for recent reviews see e.g., Husband & Schemske 1996; Dudash & Fenster 2000; Charlesworth 2003) under more or less standardised conditions, little is known about the variation of relative fitness and the susceptibility of inbred vs. outbred progeny under stressful environmental conditions or in the presence of biotic (microbial) interactions (Carr *et al.*2003).

It has been demonstrated that the microbial community may play an important role in structuring communities of higher plants (e.g., Bever, Westover & Antonovics 1997; Mills & Bever 1998; Packer & Clay 2000). Owing to the accumulation of microbes (bacteria, non-mycorrhizal fungi) with negative effects on plant performance, soil inoculated with substrate, on which the same plant species had been growing before, resulted in reduced seedling growth especially in rare species (Klironomos 2002).

The three study species of the genus *Viola* (*V. elatior*, *V. pumila* and *V. stagnina*) with a CH-CL system are among the rarest and most endangered plant species of Central Europe (Schnittler & Günther 1999). In the study area along the corridors of the rivers Rhine and Main, these species occur at the western margin of their range (Hultén & Fries 1986) and their populations are often small and partly highly isolated (Eckstein, Danihelka, Hölzel & Otte 2004). The violets are characteristic elements of species rich floodplain meadows of the alliances Cnidion and Molinion. The restoration of these meadows through transfer of plant material from species-rich source stands is the aim of extensive current conservation projects (Donath, Hölzel & Otte 2003; Hölzel & Otte 2003). Since this could lead to the establishment of new, initially small populations consisting of individuals from different source populations, the aim of the present study was to investigate possible consequences of pollen source and floral type on seed production, seed traits and offspring fitness of the study species in two different experimental environments.

We specifically addressed the following questions:

(1) Is seed production from CH flowers of the three violets reduced as a consequence of the exclusion of pollinators?
(2) Does pollen source (same flower, same population, other population) affect the number and mass of seeds per capsule and the mass per seed and are there differences in seed traits between floral types, i.e. CH and CL flowers?
(3) What is the effect of different microbial environments, i.e. soil incubated with non-sterile substrate of conspecifics ('home'-conditions) vs. sterilised substrate, and are there differences between selfed and outcrossed offspring in their response to these microbial environments?
(4) Does inbreeding depression occur with respect to vegetative growth of seedlings and does it vary between environments?

To answer these questions we carried out three separate common garden experiments, in which we excluded pollinators (Experiment I), hand-pollinated flowers with pollen from different sources (Experiment II) and planted seedlings from these hand-pollinations into two environments with different microbial communities (Experiment III).

Material and Methods

Study species

The study species and their habitats have been described in detail in Hölzel (2003) and Eckstein *et al.* (2004). In short: All three species are perennial iteroparous hemicryptophytes belonging to the section *Viola*, subsect. Rostratae (Kirschner & Skalický 1990; Quinger 1993). In Central Europe, the species mainly occur in the corridors of large lowland rivers. All three species have a Western Eurasian distribution (Hultén & Fries 1986) but *V. stagnina* even reaches the hemiboreal vegetation zone in Scandinavia. The species occur in species-rich meadows of the alliances Cnidion and Molinion that are mown once a year or along scrubby woodland fringes adjacent to floodplain meadows (only *V. elatior*). In Central Europe, *V. stagnina* is not strictly bound to floodplains but also occurs in fen meadows, tall-sedge swamps and along ditches (e.g., Pullin & Woodell 1987; Quinger 1993). All three species have a long-term persistent seed bank, which may remain viable for several years (Hölzel & Otte 2004b).

Seeds preferably germinate in spring, and seedlings develop a few leaves by the end of summer. On generative plants, both potentially cross-pollinated open (chasmogamous) flowers and obligatory self-pollinated closed (cleistogamous) flowers occur.

Origin of the experimental plant material and constraints of the experimental design

In *V. elatior* and *V. pumila*, a seed mixture from about 20 plants collected during 2001 from two large natural populations each from the Upper Rhine valley south of Frankfurt were used to create the experimental plant populations. Seeds were sown in trays and germinated seedlings potted individually in commercial soil into 12 ´12 cm pots (1000 cm3), which were kept outdoors in a common garden close to Giessen, Germany (50°32'N, 8°41.3'E, 172 m a.s.l.).

In *V. stagnina*, newly emerged seedlings were collected in 2001 from two natural populations in the Lower Main area, and potted as the other two species.

Owing to the extreme rarity of the studied violets, seed sampling had to be confined to two natural populations per species. Furthermore, low seed set and germination resulted in relatively low numbers of replicates for some of the analyses. These constraints, that are inherent in studies of rare species, lowered the power of our statistical tests and the generality of results, but on the other hand information on these rare species is urgently needed for their conservation. The pollination experiments started in spring 2002, i.e. the second year of growth, when the plants produced their first CH flowers.

Self-pollination vs. open pollination

To test the possible effects of self-pollination vs. possible cross pollination by insects on the production of CH capsules and CH seed set (Experiment I), 24 plants of similar size of each species were selected. Half of the plants were enclosed in perforated cellophane bags (Crispac bags, Baumann Saatzuchtbedarf, Waldenburg, Germany) to exclude pollinators (pollinator exclusion) and the other half were left as untreated controls. Pots were placed outdoors in a common garden, which was surrounded by meadows and watered to field capacity, if necessary. The study species do not depend on specialised pollinators. During flowering control plants were frequently visited by pollinators mainly from the orders Diptera and Hymenoptera (bees and bumblebees). After flowering, the bags were removed and all CH flowers that developed into capsules were individually wrapped into thin fabric to retain the ripe seeds following ballistic seed

dispersal. After the growing season in October, ripe capsules were harvested from each plant, brought into the laboratory. The number of seeds per capsule was counted and their bench dry mass after at least 48 h was measured. Plants from this experiment were also used for counts of CH and CL capsules over the growing season to compare the species' flowering phenology. For CH flowers, the fate of capsules was followed, i.e., using the total number of counted flowers and the number of ripe CH capsules, we analysed the proportion of capsules that failed to set seeds. Owing to the high number of CL flowers per plant, marking capsules and analysing flower abortion was not feasible for CL capsules.

Pollen source

For the analysis of possible effects of pollen source on seed number and seed mass, we hand-pollinated individual flowers of 20 plants of each species (Experiment II). The following pollination treatments were applied: pollination with (i) pollen from the same flower (**sf**, self-pollination), (ii) pollen from another plant from the same population (**sp**, potentially cross-pollinated) or (iii) from another population (**op**, cross-pollinated). Additionally, (iv) seeds from CL capsules (**cl**) were included into the analysis. This experiment was carried out in a greenhouse to exclude pollinators. Pollination was carried out by carefully removing with a pair of forceps anthers that carried ripe pollen from the donor flower and brushing these over the receptive stigma of another flower until the latter was covered with pollen. Pollen was considered ripe when anthers opened and pollen grains turned from greenish to yellow. As in other studies on the pollination biology of violets, stigmas were considered receptive by the time the petals had unfurled (e.g., Culley 2000). No evidence for delayed selfing of the study species s been observed though these mechanisms are known to occur in species of the genus *Viola* (e.g., Culley 2002). Flowers that developed into mature capsules were wrapped in thin fabric to retain the ripe seeds. We collected ripe capsules, counted the number of seeds per capsule, determined the bench dry mass of seeds of each capsule after at least 48 h and calculated the mass per seed. Seed set in *V. elatior* in this experiment was so low that the data for this species could not be analysed. Consequently, this species also had to be excluded from the subsequent experiment on offspring fitness (see below).

Offspring fitness

To test whether the effects of pollen source on offspring growth fitness differed between environmental conditions that resemble those of conspecifics (as concerns the microbial environment) and control conditions, we created two experimental environments (Experiment III). When Experiment I was finished we collected, for each species separately, the soil from the pots. In the beginning of May 2002, half of the soil was sterilised in a drying oven at 115° C for 24 h and the other half remained untreated. Then either 31.8 cm3 of sterilised or unsterilised soil (3.2% of the final soil volume) was added to new pots filled with commercial potting soil and mixed. Individual seedlings of each species, originating from seeds of different pollen source (Experiment II), were transplanted into these pots filled with sterilised or unsterilised soil of the same species. For the purpose of our experiment, pots inoculated with unsterilised soil of the same species were intended to represent the microbial environment of conspecifics ('home'-conditions), while pots with sterilised soil represented a control environment (cf. Klironomos 2002). Owing to low numbers of emerging seedlings, we could not test the growth response of *V. pumila* on soil inoculated with non-sterilised substrate, on which *V. stagnina* had been growing before and vice versa (the 'feedback approach', Bever *et al.* 1997). For *V. pumila* we did not obtain enough CL seedlings, so this source of pollination (floral type) had to be excluded from the analysis of this species.

Pots were arranged at random, kept outdoors in a common garden and watered as necessary. After eight weeks of growth the seedlings were harvested, cleaned, dried to constant mass at 70° C and weighed to the nearest milligramme.

We used data from Experiment III to estimate the level of inbreeding depression (ID) for *V. pumila* and *V. stagnina*. Relative fitness values ($RF_{(ID)}$) and the level of inbreeding depression (ID) were calculated for the final biomass of seedlings after eight weeks of growth as

$$ID = 1 - (RF_{(ID)}) = 1 - \frac{W(CH_S)}{W(CH_O)} \qquad \text{(eq. 1)},$$

where $W(CH_S)$ is the fitness of selfed CH offspring and $W(CH_O)$ the fitness of outcrossed CH offspring (cf. Culley 2000). To analyse whether fitness differences between floral types (FT) exist, we also calculated relative fitness of CL seedlings vs. selfed CH seedlings as $RF_{(FT)}$.

We determined the above indices of relative fitness and inbreeding depression for plants growing in control soil, inoculated soil and as the average of both soil treatments to test the hypothesis of higher fitness of selfed offspring under conditions that resemble those of the conspecifics ('home'-conditions; Bever *et al.* 1997) and higher fitness of outcrossed offspring under new conditions ('away'-conditions; Bever *et al.* 1997).

Statistical analysis

Flower phenology was analysed by multivariate repeated measures analysis of variance using Wilks lambda (von Ende 1993).

To analyse the single-time data from Experiments I to III, we carried out fixed factor general linear models analyses of variance (Quinn & Keough 2002). Since in experiment II not all treated capsules of each mother plant reached maturity, we could not include mother plant as a factor in our statistical analysis. Therefore, in experiment II and III we averaged the data of several capsules of each treatment per individual mother plant and, analogous to experiment I, considered each individual plant as a replicate. There were no significant effects of mother plant size on seed traits and offspring biomass. Therefore, we did not include size of the mother plant as a covariate into the statistical model. In Experiment I, we calculated a two-way ANOVA for the effects of pollination treatment (pollinator exclusion vs. open pollination) and species on the number of CH capsules and seeds per plant. In Experiment II, we did a one-way ANOVA for the effects of pollen source (cl, sf, sp, op) on the number of seeds per capsule, seed mass per capsule and mass per seed for each species separately. For significant pollen source effects, we calculated the significance of two contrasts, viz. (i) comparing selfed (sf) vs. cross-pollinated capsules (sp plus op) and (ii) CL capsules (cl) vs. capsules from selfed CH flowers (sf). Concerning the contrast analyses, we followed the recommendations in Quinn & Keough (2002) that planned contrasts should primarily test differences between groups of interest and less weight should be given to their strict orthogonality. In Experiment III, we carried out a two-way ANOVA with 'pollen source' and 'soil inoculation' as factors. We applied bootstrapping procedures to test whether relative fitness in terms of biomass deviated significantly from unity using the program Poptools (Hood 2003). This was done by randomly resampling from our data (with replacement) new biomass ratios (bootstrap sample size: 2000). Using the resampled

biomass ratios we calculated the probability that unity (i.e., a biomass ration of 1.0) was included in the random bootstrap sample distribution.

Unless otherwise stated, all statistical analyses were done using the program Statistica (ver. 6.0, Statsoft Inc, Tulsa, USA).

Results

Phenology (Experiment I)

In the experimental plant population, CH flowers started to appear in early-May. After mid-May or early June, no new CH flowers were produced (Fig. 5.1). *Viola elatior* and *V. pumila* flowered significantly earlier (approximately two weeks) than *V. stagnina* (significant species*time interaction, repeated measures ANOVA, $F_{10,402} = 8.91$, $p < 0.001$). Production of CL flowers did not overlap with the production of CH flowers (significant time*floral type interaction, repeated measures ANOVA, $F_{10,187} = 91.49$, $p < 0.001$). Cleistogamous flowers appeared in June and were produced throughout the growing season until the end of the experiment in October (Fig. 5.1). Though *V. stagnina* produced about as many open flowers as the other two species, it had two to five times more CL capsules than *V. elatior* or *V. pumila*.

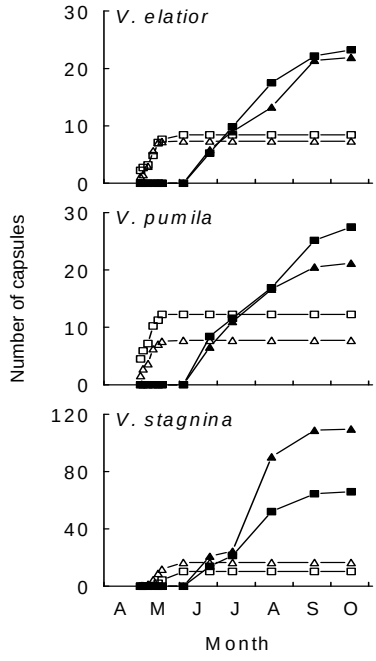

Figure 5.1 Phenology of chasmogamous flowers (open symbols) and cleistogamous capsules (filled symbols) in control (squares) and self-pollinated (triangles) plants of three species of floodplain violets.

Pollinator exclusion vs. open pollination (Experiment I)

Two-way analysis of variance revealed significant differences among species in number of ripe CH capsules and seeds ($F_{2,65}$ = 134 and $F_{2,65}$ = 75, respectively, $p < 0.001$ in both cases). There were significant species*treatment interactions for both variables ($F_{2,65}$ = 5.6 and $F_{2,65}$ = 4.4, respectively, $p < 0.02$ in both cases) but only in *V. pumila* the number of CH seeds was significantly higher in open pollinated than in pollinator exclusion plants (mean ± s.e., n = 12; 24.8 ± 10.1 vs. 2.3 ± 1.8; Tukey HSD-test, $p < 0.05$).

Not all CH flowers developed into mature capsules and fruit abortion varied among species. Relative fruit abortion (mean ± s.e., n = 12) differed significantly among species (ANOVA: F2,65 = 185, $p < 0.001$). It was much lower in *V. stagnina* (open-pollinated: 0.11 ± 0.05, self-pollinated: 0.17 ± 0.07) than in *V. pumila* (open: 0.86 ± 0.05, self: 0.97 ± 0.02) and *V. elatior* (open: 0.89 ± 0.04, self: 0.92 ±0.04). Differences between pollination treatments were marginally significant ($F_{1,65}$ = 3.34, $p = 0.072$) and there was no interaction between species and treatment ($F_{1,65}$ = 0.463, $p = 0.631$). Output of CH seeds was much lower than CL seed output in all three species.

Mass of CH seeds differed significantly among species ($F_{2,35}$ = 495, $p < 0.001$); seeds weighed about 1.75 mg in *V. elatior*, 1.45 mg in *V. pumila* and 0.60 mg in *V. stagnina*. Seed mass did not differ significantly between pollination treatments and there was no significant species*treatment interaction.

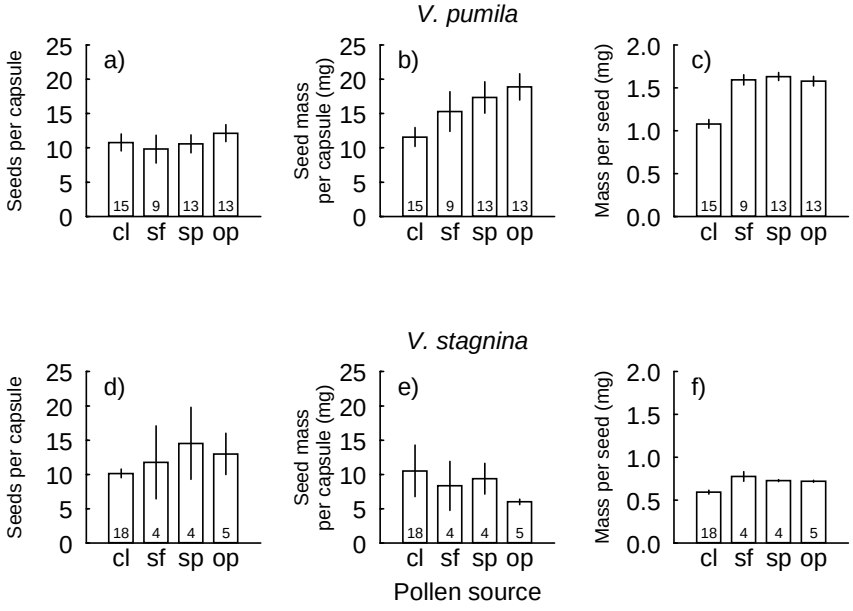

Figure 5.2 Number of seeds per capsule, seed mass per capsule (mg) and mass per seed (mg) in *Viola pumila* (upper panels, a-c) and *V. stagnina* (lower panels, d-f) in capsules differing in pollination mode and pollen source. Pollen sources: cl, cleistogamous capsules; sf, open flower pollinated with pollen from the same flower; sp, open flower pollinated with pollen from the same population; op, open flower pollinated with pollen from another population. Number of replicates are given per group above the x-axis.

Pollen source (Experiment II)

There was a significant effect of pollen source on mass per seed in *V. pumila* (Table 5.1). Seeds from cleistogamous capsules had a significantly lower mass (mean ± s.e.; 1.08 ± 0.05, n = 15) than seeds from selfed CH capsules (1.59 ± 0.06, n = 9; contrast cl vs. chs, Table 5.1, Fig. 5.2).

Also in *V. stagnina*, mass per seed was significantly lower in CL seeds (0.59 ± 0.02, n = 18) than in seeds from selfed CH capsules (0.78 ± 0.05, n = 4; Table 5.1, Fig. 5.2). Contrasting with *V. pumila*, where seed mass per capsule was highest after fertilisation with pollen from a plant of another population, in *V. stagnina* fertilisation with pollen from another population yielded the lowest seed number and mass (Fig. 5.2) though these differences were not significant.

Table 5.1 Results of an ANOVA on the effects of pollen source (cleistogamous pollination, same [chasmogamous] flower, other plant, other population) on the number of seeds per capsule (#seeds), seed dry mass per capsule (sdm) and dry mass per seed (dm per seed) of *Viola pumila* and *V. stagnina*. We carried out two planned comparisons, viz. (i) chasmogamous selfed flowers (**ch$_s$**) vs. cleistogamous flowers (**cl**) and (ii) chasmogamous selfed flowers (**ch$_s$**) vs. chasmogamous crossed flowers (**ch$_o$**).

Viola pumila		#seeds		sdm		dm per seed	
Effect	DF	MS	P	MS	P	MS	P
Pollen source	3	10.6	0.729	140.6	0.055	0.96	**<0.001**
cl vs. **ch$_s$**	1	---	---	---	---	**1.49**	**<0.001**
ch$_s$ vs. **ch$_o$**	1	---	---	---	---	<0.001	0.877
Error	46	24.7		51.7		0.035	

Viola stagnina		#seeds		sdm		dm per seed	
Effect	DF	MS	P	MS	P	MS	P
Pollen source	3	26.9	0.535	32.3	0.162	**0.056**	**<0.001**
cl vs. **ch$_s$**	1	---	---	---	---	**0.109**	**<0.001**
ch$_s$ vs. **ch$_o$**	1	---	---	---	---	0.008	0.278
Error	27	36.2		17.5		0.006	

Offspring performance (Experiment III)

In both species, *V. pumila* and *V. stagnina*, mass of seedlings after the first eight weeks of growth was significantly lower in pots where the substrate was inoculated with non-sterilised soil than in control pots where sterilised soil of the same species was added (Table 5.2, Fig. 5.3). Seedling growth was reduced by 32% in *V. pumila* and by 70% in *V. stagnina*. There was no significant main effect of pollen source and no interaction

between pollen source and soil treatment on offspring performance in any of the species (Table 5.2).

Table 5.2 Two-way ANOVA on the effects of pollen source (cleistogamous [only *V. stagnina*], same plant, same population, other population), soil inoculation (pot soil inoculated with sterilised or non-sterilised substrate in which a plant of the same species was growing before, see Methods) on the final total biomass of seedlings of two floodplain violets after eight weeks of growth.

	Viola pumila			*Viola stagnina*		
Effect	DF	MS	P	DF	MS	P
Pollen Source	2	280896	0.103	3	129692	0.594
Soil inoculation	1	**788950**	**0.015**	1	**5046742**	**0.001**
Interaction	2	55086	0.604	3	109508	0.655
Error	15	105602		16	199169	

Table 5.3 Relative fitness of offspring originating from selfed (CHs) *vs.* outcrossed chasmogamous seeds (CHo), of cleistogamous (CL) *vs.* CHs seeds and inbreeding depression of chasmogamously derived offspring in *Viola pumila* and *V. stagnina*. Fitness was estimated as the biomass ratio of seedlings of different pollination or flowering modes after 8 weeks of growth in soil, which had been inoculated with unsterilised substrate, in which plants of the same species had been growing before (microbes), with sterilised substrate (control) and using the average biomasses of both substrates (both). Inbreeding depression (ID) was calculated as ID = 1 – (Relative Fitness). Using 2000 bootstrapped biomass ratios, we tested whether relative fitness was significantly different from unity (ns, not significant ($p > 0.05$); ***, $p < 0.001$).

		Relative Fitness		***Inbreeding Depression***
	Soil	CHs *vs.* CHo	CL *vs.* CHs	1 – (CHs / CHo)
Viola pumila	control	1.42 ^{ns}	---	-0.422
	microbes	1.19 ^{ns}	---	-0.190
	both	1.33 ^{ns}	---	-0.326
Viola stagnina	control	0.86 ^{ns}	1.25 ^{ns}	0.144
	microbes	2.09***	0.64 ^{ns}	-1.093
	both	1.08 ^{ns}	1.04 ^{ns}	-0.076

In *V. pumila* relative fitness of selfed CH offspring was higher than that of outcrossed CH offspring in both soil treatments (Table 5.3, relative fitness (CHs vs. CHo) > 1). The ratio did not differ significantly from unity, which suggested that there was no inbreeding depression with respect to biomass. In contrast, relative fitness in *V. stagnina* changed considerably with soil treatment (Table 5.3): selfed CH offspring performed

significantly better ($p < 0.001$) than outcrossed CH offspring when growing with the soil microbial community of conspecifics, but performance was similar to that of outcrossed CH offspring on control soils (inoculated with sterilised soil of the same species). Depending on the soil treatment the relative fitness of selfed offspring changed between floral types but the ratios did not deviate significantly from unity. However, relative fitness averaged across soil treatments did not differ between selfed and outcrossed CH offspring and between CL and selfed CH progeny (Table 5.3).

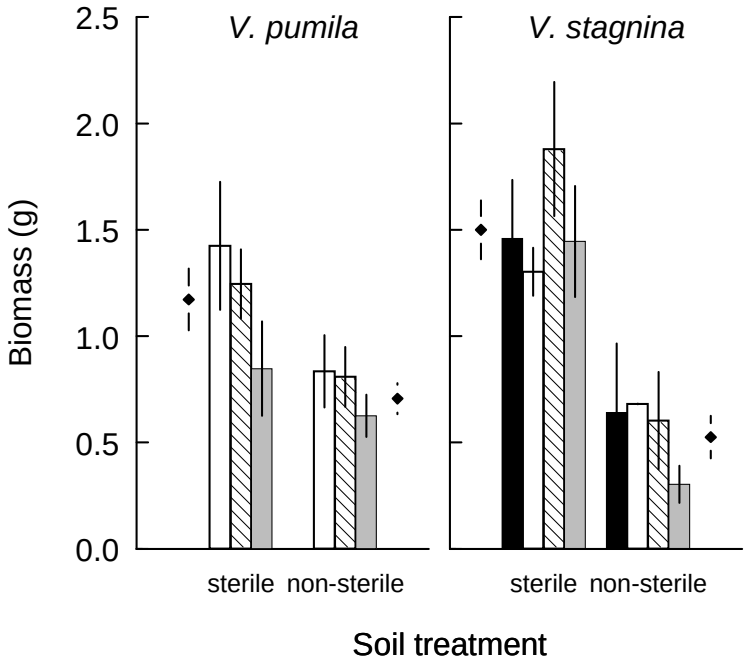

Figure 5.3 Total biomass after 8 weeks of plants of *Viola pumila* and *V. stagnina* originating from cleistogamous seeds (black bars [only *V. stagnina*]) or seeds of open flowers fertilised with pollen from the same flower (white bars), the same population (hatched bars) or an other population (grey bars). Plants were grown in pots in peat soil that was inoculated with heat-sterilised (sterile) or non-sterilised (non-sterile) substrate where plants of the same species had been growing before (see Methods). Data are means per mother plant ± s.e. (n = 2 to 7). Diamonds denote average biomass per soil treatment across pollen sources ± s.e. (n = 9 to12).

Discussion

All three violets studied belong to a guild of species within the floodplain communities that flower in early spring. However, *Viola elatior* and *V. pumila* appear to consistently flower about one week earlier than *V. stagnina* (Fig. 5.1). This was also found in field populations (Göbel 2001). The production of CH and CL capsules was temporarily separated in the study species, with CL capsules emerging after cessation of CH flowering. It is very likely that production of the different floral types is triggered by different environmental clues (Le Corff 1993) and that species with non-overlapping production of floral types may regulate the production of CL capsules according to the reproductive success of the CH flowers (Redbo-Torstensson & Berg 1995).

Pollinator exclusion had little effect on the production of CH capsules and seeds in the study species. Abortion of CH capsules was about 90% in *V. elatior* and *V. pumila*, while CL capsules produced abundant seeds. Therefore, seeds from CH flowers may contribute only a small fraction of total seed production, but may be more important in certain years. The fact that mass per seed of *V. pumila* estimated using capsules from field populations (Hölzel & Otte 2004a) were identical with the values for CL seeds from the present study (1.08 mg in both cases) suggests that the majority of ripe seeds in natural populations may be from CL pollination. This conclusion is further supported by results from field populations of the study species in the Czech Republic, which indicate that numbers of CL capsules producing seeds outweigh CH capsules and that CL reproduction is safer than open flowering (B. Lučeničová, J. Danihelka, unpubl. results).

To our knowledge, the present paper is one of a few studies that explicitly addressed the ecological consequences of pollen source for seed traits and offspring fitness in CH-CL systems by separating pollination mode (selfing vs. crossing) and differences between floral types (ch vs. cl) (cf. Culley 2000). Usually, CH flowers have simply been assumed to be outcrossed (Waller 1984; Mitchell-Olds & Waller 1985; Clay & Antonovics 1985; Berg & Redbo-Torstensson 1999, 2000), though selfing rates of CH flowers may be high in CH-CL species (Lesica; Learly; Allendorf; & Bilderback 1988; Cole & Biesboer 1992; Culley 2002; Berg 2003).

In both species, seedling growth was significantly reduced under 'home'-conditions (Table 5.2, Fig. 5.3). This effect, which most probably is owing to negative effects of the microbial soil community, has been demonstrated for a large number of plant species (Bever 1994; Packer & Clay 2000; Klironomos 2002). Though we did not find general interactions between pollen source and soil treatment (Table 5.2), our results still suggest that under stressful conditions (i.e., with a conspecific microbial community) relative fitness of selfed offspring of *V. stagnina* was significantly higher than that of crossed progeny (Fig. 5.3, Table 5.3). Recombination between distant populations apparently resulted in genotypes that showed signs of outbreeding depression under stressful conditions. As a consequence, the mode of pollination may have some effect on the outcome of biotic interactions but it was only recently that studies have begun to address this topic (Carr *et al.* 2003).

The finding that performance of CL offspring of *V. stagnina* under 'home'-conditions was considerably lower than that of selfed CH seedlings (Table 5.3, Fig. 5.3) demonstrates that separation of differences between pollination modes and floral types is essential in CH-CL systems (Culley 2000). Since CL seeds have a significantly lower mass than CH seeds (Fig. 5.2), the observed difference in offspring fitness may not be related to genetic reasons, but a consequence of floral type differences (Culley 2000). In the European *Viola hirta*, CL seeds had significantly lower mass per seed than CH seeds, but there were no differences between floral types in *V. mirabilis* and *V. riviniana* (Berg & Redbo-Torstensson 1999). Individual seed mass of CL seeds in the North-American *V. canadensis* was even higher than that of CH seeds (Culley 2000). This was primarily caused by a large elaiosome in CL seeds, which is lacking in CH seeds of *V. canadensis* (Culley, pers. comm.). Mass of individual seeds will be constrained by the amount of resources allocated to reproduction and/or the environmental conditions at the time of seed set. Therefore, mass per seed in our study species may be lower in CL seeds than in CH seeds because of a trade-off between seed number and size. Assuming the same amount of resources for both types of capsules, much more CL than CH seeds are produced and the pure quantity of CL seeds will outweigh the potentially lower costs for CL capsules (no petals, no nectar). In *V. elatior* larger CL seeds had better germinability (Eckstein & Otte 2004), but for *V. pumila* and *V. stagnina* there is no information on, whether smaller seed mass of CL seeds will have any effects on e.g., seed survival, persistence, germinability and seedling growth. However, there was observational evidence that germination was lower in CL seeds than in CH seeds from our experimental plants and germination percentages were lower in CL than in CH seeds in other species of *Viola* and in *Oxalis acetosella* (Berg & Redbo-Torstensson 1999, 2000). In *Impatiens capensis*, seed mass was lower in CL seeds than in CH seeds when all seeds were considered but not for germinated seeds (Mitchell-Olds & Waller 1985), which suggests lower germination of smaller CL seeds.

In accordance with our results, the number of seeds per capsule did not differ between pollen sources (sf, sp, op); in a large population of *Pedicularis palustris*, however, autogamous selfing in a small population resulted in reduced seed production (Karrenberg & Jensen 2000). In three European violet species there were no differences in seed abortion between CH and CL capsules (Berg & Redbo-Torstensson 1999), but it is not clear whether CH flowers were actually cross-pollinated in that study.

Judging from seedling biomass reached after eight weeks, there was no indication of inbreeding depression in the study species (Table 5.3). In *V. stagnina*, relative fitness varied between soil treatments, but on average fitness did not differ between pollination modes or floral types (Table 5.3). Similarly, no indication of inbreeding depression in three other *Viola* species was found by Berg & Redbo-Torstensson (1999). This may suggest that deleterious alleles that may become homozygous as a consequence of selfing have been purged out of the populations during the phylogenetic history of the study species. However, early inbreeding depression was not related to population isolation in *Swertia perennis* (Lienert & Fischer 2002) which demonstrates that early inbreeding depression was not purged out from strongly isolated populations. Overall relative fitness was higher in CH than in CL seedlings of *Impatiens capensis* but there were significant differences between years, traits and populations (Waller 1984). However, in the latter studies on *Viola* and *Impatiens* it was not clear whether CH flowers were self- or cross-pollinated. Similarly, in *Viola canadensis* relative fitness of selfed and outcrossed offspring varied among plant traits but inbreeding depression and differences between floral types were generally low (Culley 2000).

The fact that *V. stagnina* plants pollinated with pollen from another population had a significantly lower relative fitness than plants pollinated by pollen of the same population under 'home'-conditions (Table 5.3, Fig. 5.3) suggest that there may be outbreeding depression among distant populations. Indications for outbreeding depression were also found for *Gentianella germanica*, where crosses within the same population performed better than inter-population crosses (Fischer & Matthies 1997). When experimental crosses along a gradient in population distance are carried out, often both inbreeding depression, outbreeding depression and a 'optimal outcrossing distance' can be found (Waser & Price 1994; Schierup & Christiansen 1996; Fischer & Matthies 1997).

Since populations of *V. stagnina* are highly isolated in the study area (Eckstein *et al.* 2004), gene flow is very low and consequently genetic differentiation among populations rather high (Eckstein *et al.* unpubl.). Therefore, crossings between these populations may lead to biochemical or physiological incompatibilities between genes or the breaking of coadapted gene complexes (Dobzhansky 1972; Siikamäki 1999) of these populations and to lowered fitness especially under stressful conditions. Outbreeding depression is of great concern in conservation biology where efforts are

undertaken to restore genetic diversity and heterozygosity of rare plant species (Dudash & Fenster 2000; Fenster & Galloway 2000; Montalvo & Ellstrand 2001; Edmands & Timmermann 2003). Our data suggest that the creation of initially small founder populations through the transfer of plant material in restoration projects, which may lead to increased probabilities of selfing, will not directly affect population viability of these rare species. However, in new populations consisting of genotypes from different source stands, outbreeding depression as the result of cross-pollination may impair seedling growth and population viability. Therefore, mixing the donor material from different populations should be avoided.

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Chapter 6

Genetic structure among and within peripheral and central populations of three endangered floodplain violets

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Abstract

Understanding the partitioning of genetic variance in peripheral and central populations may shed more light on the effects of genetic drift and gene flow on population genetic structure and, thereby, improve attempts to conserve genetic diversity. We analysed genetic structure of peripheral and central populations of three insect-pollinated violets (*Viola elatior*, *V. pumila*, *V. stagnina*) to evaluate to what extent these patterns can be explained by gene flow and genetic drift. Amplified fragment length polymorphism was used to analyse 930 individuals of 50 populations. Consistent with theoretical predictions, peripheral populations were smaller and more isolated, differentiation was stronger, and genetic diversity and gene flow lower in peripheral populations of *Viola pumila* and *V. stagnina*. In *Viola elatior*, probably historic fragmentation effects linked to its specific habitat type were superimposed on the plant geographic (peripheral-central) patterns, resulting in lower relative importance of gene flow in central populations. Genetic variation between regions (3-6%), among (30-37%) and within populations (60-64%) was significant. Peripheral populations lacked markers that were rare and localised in central populations. Loss of widespread markers in peripheral *Viola stagnina* populations indicated genetic erosion. Autocorrelation within populations was statistically significant up to a distance of 10-20 m. Higher average genetic similarity in peripheral populations than in central ones indicated higher local gene flow, probably owing to management practices. Peripheral populations

contributed significantly to genetic variation and contained unique markers, which made them valuable for the conservation of genetic diversity.

Introduction

The abundance and density of individuals and population frequency are not constant across a species range but usually decrease towards the range margin (Lawton 1993, Lesica & Allendorf 1995, '*abundant centre hypothesis*', cf. Sagarin & Gaines 2002). Range margin populations can be geographically or ecologically peripheral (Lesica & Allendorf 1995). In many cases the ecological conditions in peripheral populations will be different from those in central populations. Although the study of species ranges and the analysis of causes for distribution limits at the range margin have traditionally been a topic of plant geography, patterns and ecological processes at the range margin have also received attention from plant ecologists (e.g. Carter & Prince 1981, Nantel & Gagnon 1999, Kluth & Bruelheide 2005), conservationists and plant geneticists (e.g. Lesica & Allendorf 1995 and references therein, Durka 1999, Lammi *et al.* 1999, Van Rossum *et al.* 2003).

Low habitat quality at the range margin may affect species performance and reduce reproduction and dispersal (Pigott & Huntley 1981, García *et al.* 2000, Dorken & Eckert 2001). Additionally, the habitat type or safe-sites for germination may be infrequent at the range margin (Dinsdale *et al.* 2000, Jump & Woodward 2003). Therefore, peripheral plant populations will often be (i) more isolated (Lawton 1993, Lesica & Allendorf 1995) and (ii) contain fewer individuals than central populations (Durka 1999, Lammi *et al.* 1999; but see Kluth & Bruelheide 2005). Small populations face an increased risk of extinction through environmental stochasticity or catastrophes (Lande 1993, Menges & Dolan 1998). The viability of these populations may also be reduced because of the increased chance of mating between relatives (Menges 1991, Fischer & Matthies 1998). Additionally, small isolated populations may suffer from pollinator limitation (Jennertsen 1988, Ågren 1996). Low habitat quality and/or small population size at the range margin lead to increased variability of demographic rates (Nantel & Gagnon 1999). This may lead to higher rates of extinction, while larger distances between suitable habitats and source populations reduce the rate of re-colonisation of empty habitats, lowering the overall proportion of occupied patches.

A higher degree of isolation at the range margin will have similar consequences as fragmentation (Young *et al.* 1996). Habitat destruction and land-use changes, which result in population isolation through fragmentation, may severely influence gene flow at the landscape scale (Manel *et al.* 2003). These anthropogenic effects also occur in the core of a species' distribution and may hence be superimposed on the plant geographic (core-periphery) patterns.

The expected population genetic consequences of small population size and isolation are (i) reduced genetic diversity of peripheral populations due to founder effects, bottlenecks, inbreeding, genetic drift, or directional selection and (ii) increased differentiation among populations through reduced rates of gene flow (Lesica & Allendorf 1995, Durka 1999, Hutchison & Templeton 1999, Lammi *et al.* 1999). Even under similar selection in central and peripheral populations, isolation will increase genetic divergence at the range margin (Cohan 1984). Consequently, geographically peripheral populations may differ considerably from core populations and hence contribute significantly to geographic variation (Durka 1999). Isolation and directional selection in peripheral populations, which support genetic divergence, may promote speciation at the boundaries of the species range (Lesica & Allendorf 1995). Peripheral populations may be especially important for the conservation of genetic variation *per se* (Lesica & Allendorf 1995, Van Rossum *et al.* 2003) and for conservation in the light of global change since they may contain genotypes evolved under variable, extreme, and/or suboptimal conditions (Safriel *et al.* 1994).

If differences in the rates of mutation and selection between core and peripheral populations can be ignored, the *relative* role of genetic drift and gene flow for shaping the regional population structure can be analysed through the relationships between genetic (F_{ST}) and geographic distances. This approach of Hutchison and Templeton (1999), is based on a stepping-stone model of population structure, i.e. a model in which gene flow is most likely between neighbouring populations. A pattern consistent with equilibrium between gene flow and drift, i.e. isolation by distance (Wright 1931), should be characterised by a positive monotonic relationship between genetic and geographic distance (cf. Fig. 1a, case I). Because of the homogenising effect of gene flow, populations at closer distances should not only be separated by smaller genetic distance but also the variation in genetic distance should be lower. As geographic distances increase the relative effect of gene flow decreases and widely separated populations are

both genetically more distant and genetic distance shows larger variation due to genetic stochasticity. The assumption of equilibrium conditions implicit in the isolation-by-distance model is often not met in natural populations. This may be because the populations and/or the required conditions have not been present long enough to achieve equilibrium patterns of isolation by distance (McCauley 1993). The expected patterns under non-equilibrium conditions are affected by (i) the time a region has been occupied (historical effects) and (ii) the degree to which regional dispersal is limited through fragmentation (contemporary effects). If, for example, a region has been colonised after the last glaciation from relatively homogeneous source populations in glacial refugia, the established populations will be genetically relatively similar and genetic and geographic distance will not be correlated (cf. Fig. 1a, case II). If gene flow remains relatively strong in comparison with random genetic drift this patterns will persist. Therefore, under non-equilibrium conditions small variation in F_{ST} indicates higher *relative* importance of gene flow over genetic drift (Hutchison & Templeton 1999). However, if environmental conditions lead to fragmentation and isolation of populations across the whole region genetic drift will become more influential and variation in F_{ST} will increase (Fig. 1a, case III).

Empirical data on the differential influence of genetic drift and gene flow on regional genetic structure in core and range margin populations are strongly biased towards wind-pollinated woody plants, mainly coniferous trees (Lesica & Allendorf 1995, Gapare *et al.* 2005, and references in these papers). However, relatively little is known about insect-pollinated perennial herbs (but see Dolan 1994, Durka 1999, Lammi *et al.* 1999, Van Rossum *et al.* 2003), which owing to their pollination and mating system - allowing gene flow only over relatively short distances - may suffer more from isolation and fragmentation than wind-pollinated plant species.

Viola elatior Fries, *V. pumila* Chaix, and *V. stagnina* Kit. (syn. *V. persicifolia*) are very rare and endangered in Central Europe and red-listed in many European countries (Schnittler & Günther 1999). They have become rare and endangered through melioration and fragmentation of their habitats. Owing to their red-list status, populations of the study species have been the focus of intensive floristic inventories, monitoring programs and conservation biological studies in the two study regions (e.g., Hölzel 1999, 2003, Sumberová *et al.* 2000, Eckstein *et al.* 2004, 2006a). Although a few populations may have been overlooked, the numbers and locations of all recently extant

populations of the study species are exceptionally well documented. Populations in the floodplains of the lower Dyje River in the vicinity of Břeclav (Czech Republic) are within the main range of the species. Populations along the Upper Rhine south of Frankfurt (Germany) are situated at the periphery of the species ranges, separated from the main distribution area by about 600 kilometres. Since the species occupy similar habitats as along the Dyje River, it is very likely that peripheral populations of the study species are geographically but not ecologically peripheral. These species belong to the section *Viola*, subsection *Rostratae* (Kirschner & Skalický 1990). They have continental distribution ranges with centres of occurrence in the temperate zone of Eastern Europe and Western Siberia (Hultén & Fries 1986). In Central Europe the species reach their western range margin and show strong affinity to the valleys of large lowland rivers (Burkart 2001). These violets are iteroparous hemicryptophytes with a complex life-cycle, a mixed mating system with chasmogamous and cleistogamous flowers ('true' cleistogamous species, Plitmann 1995) and a persistent seed bank (Hölzel & Otte 2004). *Viola pumila* and *V. stagnina* occur mainly in species-rich, regularly managed floodplain meadows and wet grasslands, whereas *V. elatior* is typical of alluvial woodland fringes and other ecotonal habitats bordering floodplain meadows. Details on the taxonomy, habitat requirements, distribution, and population biology of the study species are given in Hölzel (2003) and Eckstein *et al.* (2004, 2006a).

The main aims of our research were to analyse the local and regional genetic structure of these congeneric endangered insect-pollinated grassland herbs in order to evaluate the relative importance of genetic drift and gene flow in central and peripheral populations. We tested the following predictions of the abundant centre hypothesis (Lawton 1993, Sagarin & Gaines 2002):

(1) Peripheral populations of these species are smaller and more isolated than central ones.

(2) Owing to larger effects of random genetic drift in peripheral populations these are characterised by lower genetic diversity and stronger genetic divergence among populations.

We further asked whether there is spatial genetic structure in central and peripheral populations at the local scale, and whether peripheral populations contain unique

genetic markers not present in core populations, which would make them valuable for conservation from a population genetic point of view.

Materials and Methods

Study regions

The study was carried out in two regions that represent two strongholds of the study species in Central Europe (Hölzel 2003). The Upper Rhine region is densely populated and the landscape fragmented through settlements, roads, and large areas of intensive farming. Similarly, also in the Dyje region north-west of Břeclav, intensive crop fields prevail outside the floodplain, whereas directly in the floodplain, forests and relatively un-intensively managed meadows have been preserved. The region south of Břeclav is only very sparsely populated, and alluvial forests, surrounding large and middle-size patches of floodplain meadows, dominate the landscape (Grulich *et al.* 2000).

Field sampling and data collection

Within each region we selected seven (Germany) to ten (Czech Republic) populations that occurred in the characteristic vegetation types and covered the main regional distribution of the species. A population consisted of those conspecific individuals that occurred within the same grassland allotment as the basic units within the cultural landscape receiving identical land-use management. Populations were separated by at least 150 m. *Viola pumila* and *V. stagnina* occur mostly as patches of scattered individuals within the floodplain meadows. Though their populations may consist of hundreds or thousands of individuals, their spatial extent within a habitat is restricted. This is also true for *V. elatior*, which often grows in linear habitats (Eckstein *et al.* 2006a). Since populations of the species (except for *V. stagnina* in Germany) were aggregated in two areas, we adopted a stratified sampling design to assure the inclusion of populations from both areas (Appendix 1); because of technical problems one German population of *Viola pumila* had to be omitted. We collected samples from about 37% (*V. elatior*, *V. stagnina*) and 17% (*V. pumila*) of all known extant populations in both study regions.

From digital maps we obtained the geographic coordinates of all extant populations to calculate (for each population) the distance to the nearest conspecific population as a measure of isolation. This measure of isolation has two advantages over, e.g., the

average pairwise distance. Firstly, it nicely matches the stepping-stone model of Hutchison and Templeton (1999), where gene flow is most likely between neighbouring populations and therefore distance to the nearest population is more important than the average distance of all pairs of populations. Secondly, we obtained an independent value for each population, which makes this measure more appropriate for univariate inferential statistics (e.g., analysis of variance) than pairwise distances.

Additionally, we estimated population size (number of plants except seedlings) of all sampled populations on a logarithmic scale, i.e. 1-100 (= 1), 101-1000 (= 2), and 1001-10,000 (= 3) individuals by walking line transects across populations.

Within each population a line transect was laid out, along which a maximum of 20 plants were randomly selected and their coordinates (x: distance along the transect, and y: distance perpendicular to the line, distance between two sampled plants: >0.1 m) recorded. In populations with <20 individuals tissue samples of all individuals were taken. The youngest shoot tip of each individual was sampled, stored in a paper bag and dried at room temperature. Samples were brought to the laboratory for DNA extractions as fast as possible. During DNA extraction and further processing a few samples were lost, but in total we analysed 930 individuals from 50 populations (Appendix 1).

DNA Extraction and AFLP analyses

Protocols for DNA extraction and AFLP analyses followed those described in detail by O'Neill (2005) for *Viola arvensis*. Briefly, DNA was extracted according to Doyle and Doyle (1987). Dried leaf material frozen with liquid nitrogen was crushed before being transferred into cetyl-trimethylammonium bromide (CTAB) extraction buffer and incubated at 65 °C for 30 min. After two washes with a chloroform:isoamylalcohol mix (24:1) and centrifugation, sodium acetate and ammonium acetate were added to the supernatant. Iso-propylalcohol was added to precipitate the DNA, and after further centrifugation the remaining pellet was washed with 70% ethanol containing ammonium acetate. After drying the pellet was resuspended in 100 µL TE buffer with added RNAse.

AFLP analysis was performed essentially as described by Vos *et al.* (1995), using AFLP Core Reagent Kits (Gibco Life Technologies, Karlsruhe, Germany). The DNA of each sample was digested with the restriction enzymes MseI and EcoRI in a volume of 25 µL containing reaction buffer at 37 °C for two hours , followed by a final step of 70 °C for 15

min. Both the +1 and +3 selective amplification began with a three minute 94 °C denaturation and ended with a five minute 72 °C polymerization. For the +1 amplification, the denaturation (94 °C, 30 sec), annealing (56 °C, 1 min), and polymerisation (72°C, 1 min) cycle was repeated twenty times. For the +3 amplification two sets of cycles were carried out. The first consisted of 12 cycles of denaturation (94 °C, 30 sec), annealing (65 °C, 30 sec), and polymerisation (72 °C, 1 min), with the annealing temperature decreasing by -0.7 °C per cycle. Following these cycles, the PCR process continued with a of denaturation (94 °C, 30 sec), annealing (56 °C, 30 sec), and polymerisation (72 °C, 1 min) cycle repeated 23 times.

The following four +3-primer combinations were used for all species: (1) EcoRI-ACT / MseI-ACG, (2) EcoRI-ACT / MseI-ACT, (3) EcoRI-ATC / MseI-AGG and (4) EcoRI-ATC / MseI-ATT.

Gel Electrophoresis

The amplification products from AFLP analyses were visualised through the use of a 0.2 mm thick, 25 cm long polyacrylamide gel (based on an 8% Long Ranger Gel Solution) in a Li-COR Gene Readir 4200 DNA sequencer (Li-COR Inc, Lincoln, USA). All products were mixed with a STOP loading buffer at a 1:1 ratio before being denatured at 94 °C for 3 min. Reverse primers of +3 EcoRI-primers for AFLP analysis were fluorescently labelled with IRD800.

With a laser emitting a wavelength of 800 nm this dye is excited and fluoresces, allowing the DNA to be detected. A 1x TBE buffer was used for running the gels. Standard parameters were used for the separation of fragments: 1500 V, 50 W, 35 mA, 48 °C. The size (in base pairs) of bands appearing on the gel were determined by comparison to a 50-350 bp molecular size standard (Li-COR) run on both edges of the gel. AFLP products were scored visually as the presence (1) or absence (0) of unambiguous AFLP bands. All samples were scored by the same person.

Calculations and data analyses

The effects of species, region, and their interactions on the population size and on isolation (i.e. distance to the nearest conspecific population) were tested in a two-way fixed effect permutation analysis of variance (Quinn & Keough 2002).

We used two estimates of genetic diversity. One was based on allele frequencies, i.e. an estimator of expected heterozygosity (H_e) according to Lynch and Milligan (1994, *gene diversity*). The other was based on the number of pairwise differences in banding patterns within populations divided by n-1 (where n is the number of samples, cf. Fischer & Matthies 1998, *molecular variance*). Gene diversity was calculated after the estimation of allelic frequencies using a Bayesian method with non-uniform prior distribution of allele frequencies (Zhivotovsky 1999) implemented in the program AFLP-SURV (Vekemans *et al.* 2002). This method gives accurate unbiased estimates of null allele frequencies in dominant marker systems (Zhivotovsky 1999; Kraus 2000). To account for the mixed mating system of the study species (Eckstein & Otte 2005) and because selfing rates in violets may vary dramatically between years (Culley 2002), we assumed a selfing rate of 0.5, i.e. an F_{is} of 0.33, in all calculations. However, assuming either Hardy-Weinberg equilibrium for the populations (i.e., using F_{is}=0 in the calculations) or highly selfing populations (F_{is}=0.9) had very little effect on the results (a difference of 6 and 13%, respectively, as compared with F_{is}=0.33) and did not change the general conclusions.

First, a three-level hierarchical analysis of molecular variance (AMOVA) was calculated with the program ARLEQUIN ver. 3.0 (Excoffier *et al.* 2005). We analysed the following levels for each species-region combination: (i) region, (ii) populations within region, and (iii) individuals within populations within regions. Additionally, a two-level AMOVA was calculated for each species-region combination to analyse the partitioning of molecular variance among and within populations.

Since AFLP markers are dominant, of the two 'phenotypic' states of a polymorphic marker (0/1) only one produces a band on the gel. We classified the visible bands of the AFLP markers (i.e. only the dominant state) according to their occurrence in populations as widespread (occurring in ≥25% of the populations) or localised (<25%), and according to their average frequency across populations as common (average frequency ≥0.05) or rare (<0.05). This approach is similar to the two-way classification of alleles developed by Marshall and Brown (1975). By restricting the analysis to only one state the estimated occurrences and frequencies produced unbiased independent data. We obtained contingency tables for core and peripheral regions of each species that could be compared using a χ^2-test.

We calculated a Mantel-test (Legendre & Legendre 1998) for each species-region combination, using F_{st}-values obtained from AFLP-SURV to test for significant correlation between pairwise genetic and geographic distances. Monotonic increasing genetic distance with geographic distance would indicate case I (Fig. 1a), i.e. an equilibrium between genetic drift and gene flow. For each species and region we analysed the scattergram of F_{st} *versus* geographic distance to infer the relative influences of gene flow and drift on the distribution of genetic variability following Hutchison and Templeton (1999). We explicitly tested, whether average pairwise F_{st} was higher in peripheral than in central populations using two-sample Monte Carlo tests (Manly 2001). Additionally, we resampled the variance ratio in F_{st} between peripheral and central populations to test whether the scatter in F_{st}-values was significantly higher in peripheral populations.

To test for the presence of small scale genetic structure within populations, we used geographic distances and Sørensen similarity index, which is similar to the Dice-index (Legendre & Legendre 1998), based on the presence/absence of AFLP markers among pairs of individuals within populations. For each species and region we constructed a matrix **S** that contained the pairwise genetic similarity between all individuals in all populations and a matrix **D** with the respective geographic distances. We computed multivariate Mantel correlograms (Legendre & Legendre 1998: 736ff) by coding in **D** all distances of a certain predefined distance class by 1 and all other distances (of all within- and among-populations pairs) by 0 to obtain the model matrix $\mathbf{X}_1$. Different model matrices $\mathbf{X}_d$ were prepared for all distance classes **d**. Distance classes were chosen to comprise roughly similar numbers of pairs. Then a Mantel-test was calculated between **S** and each of the model matrices using the normalized Mantel statistic (r_M). This approach is the multivariate pendant to autocorrelation analysis on univariate quantitative data using e.g. Moran's I (Legendre & Legendre 1998). Significant deviation of r_M from zero was tested for each distance class by a permutation approach. We applied progressive Bonferroni correction (Legendre & Legendre 1998) to account for multiple testing. Mantel tests were calculated with the program PC-ORD 4 (McCune & Mefford 1999) using 9999 permutations.

Results

Consistent with our expectation peripheral populations were significantly smaller than central populations, and this pattern did not differ among species (Table 1). Across species, peripheral populations were significantly more isolated than central ones. The largest average distance to the nearest population was found in peripheral populations of *V. stagnina* (Table 2). In contrast to our working hypothesis, peripheral populations of *V. elatior* were significantly less isolated than core populations (significant species-region interaction).

AFLP analyses produced 160 scorable bands, 75% of which were polymorphic. We recorded a total of 90, 155, and 162 AFLP phenotypes in *Viola elatior*, *V. pumila*, and *V. stagnina*, respectively. For further analyses, we prepared three data sets, one for each species, based on the AFLP markers present in each of the three species (*V. elatior*: 152 in total/97 polymorphic markers; *V. pumila*: 155/108; *V. stagnina*: 156/113). Percentage polymorphism across regions ranged from 63 to 72%. There was no indication of size homoplasy in the data sets because no significant negative correlation between fragment size and frequency was found (Vekemans *et al.* 2002). The numbers of markers and percentage of polymorphic markers per population were significantly higher in central populations of *Viola pumila* and *V. stagnina* but did not differ in *V. elatior* (Table 3). Similarly, gene diversity and molecular variance, both measures of genetic diversity, were higher in central than in peripheral populations in *Viola pumila* and *V. stagnina* (Table 3). There was no significant difference in *Viola elatior*.

Table 6.1 Results of a permutation analysis of variance on the effects of species and the location of populations with respect to the species range (peripheral vs. central) on the size of the populations sampled for the genetic study and on the isolation (distance to the nearest conspecific population) of all known extant populations. Abbreviations: df, degrees of freedom; MS, mean square; P, proportion of permutations, where the randomised F-value was larger than the original F-value (number of permutations: 10,000).

	Population size			Isolation		
Source of variation	**Df**	**MS**	***P***	**df**	**MS**	***P***
species (S)	2	3.61	0.0001	2	58.31	0.0001
region (R)	1	2.34	0.0094	1	72.27	0.0001
S * R	2	0.21	0.5040	2	70.75	0.0001
model	5	2.15	0.0001	5	43.83	0.0001
error	46	0.30		177	1.18	
total	51	0.48		182	2.35	

Three-level analysis of molecular variance revealed that all variance components were significant and that partitioning of molecular variance was similar in the three study species (data not shown). Three to 6% of the variation was found between regions, 30 to 37% among populations within regions and 60 to 64% among individuals within populations. Two-level analyses for each species-region combination showed that partitioning of molecular variance between and within populations was similar in peripheral and core populations (37.0 *vs*. 39.1%, respectively) of *Viola elatior*, while in *V. pumila* (48.8 *vs*. 24.3%) and *V. stagnina* (50.7 *vs*. 25.9%) divergence was higher in peripheral than in core populations.

Table 6.2 Average distance to the nearest conspecific population for *Viola elatior*, *V. pumila*, and *V. stagnina* located at the periphery (Upper Rhine, Germany) or at the centre (Dyje, Czech Republic) of their ranges.

Species	**Region**	**Mean (km)**	***n***	***P***
V. elatior	Dyje	1.07 ± 0.22	26	0.0077
	Upper Rhine	0.42 ± 0.07	19	
V. pumila	Dyje	0.52 ± 0.06	57	0.0092
	Upper Rhine	0.97 ± 0.21	35	
V. stagnina	Dyje	0.57 ± 0.10	34	0.0001
	Upper Rhine	5.01 ± 0.88	12	

Data are means ± s.e., *n* is the number of known extant populations in the study areas and *P* gives the proportion of permutations, where the randomised F-value was larger than the original F-value (one-way permutation GLM ANOVA, number of permutations: 10,000).

The distributions of markers into different classes based on occurrence and frequency differed significantly between core and peripheral regions in *Viola elatior* and *V. stagnina* (Table 4). Peripheral populations of *Viola elatior* had fewer common but localised markers, while they contained more rare widespread ones than central populations. In *V. stagnina* these differences between regions were diametrically opposed. Similarly, more unique AFLP markers were present in peripheral populations of *Viola elatior* than in core populations but the pattern was reversed in *V. stagnina*. In peripheral populations of *Viola elatior*, *V. pumila*, and *V. stagnina* one, two, and eight rare localised markers were absent, respectively. Additionally, peripheral populations of *Viola stagnina* lacked one rare widespread marker and three common widespread markers that occurred in ≥40% of the core populations and had an average frequency of ≥0.1120.

Peripheral populations contained one unique common widespread marker in *Viola elatior* (frequency 0.0563/occurrence 28.6%), one in *V. pumila* (0.0515/33.3%), and one common localised marker in *V. stagnina* (0.1612/16.7%).

Table 6.3 Gene diversity, molecular variance, number of markers (# markers) and percentage polymorphic markers within central (Dyje floodplains, Czech Republic) and peripheral (Upper Rhine, Germany) populations of the three studied violets. For estimation of gene diversity a selfing rate (*s*) of 0.5*, i.e. a F_{IS} of 0.33, was assumed (see Methods). *P*-values are from a permutation t-test (10,000 permutations). Data are mean ± s.e.

Species	**Region**	***n***	*Gene diversity**	*P*	*Molecular variance*	*P*	*# loci*	*P*	*Polymorphic loci (%)*	*P*
Viola elatior	Dyje	10	0.1146 ± 0.0066	0.4189	5.03 ± 0.69	0.7939	122.9 ± 2.0	0.3657	19.6 ± 2.8	0.3936
	Rhine	7	0.1121 ± 0.0141		5.35 ± 1.02		126.1 ± 2.9		20.8 ± 3.0	
Viola pumila	Dyje	10	0.1735 ± 0.0059	0.0002	10.59 ± 0.56	0.0001	129.0 ± 0.8	0.0185	42.2 ± 1.4	0.0001
	Rhine	6	0.1164 ± 0.0072		6.20 ± 0.56		124.3 ± 2.0		25.9 ± 1.2	
Viola stagnina	Dyje	10	0.1364 ± 0.0070	0.0276	8.06 ± 0.54	0.0160	113.2 ± 1.4	0.0260	40.5 ± 2.8	0.0151
	Rhine	7	0.1129 ± 0.0083		6.11 ± 0.56		108.9 ± 1.1		30.5 ± 3.0	

*When assuming either Hardy-Weinberg equilibrium (*s*=0.0) or highly selfing populations (*s*=0.95) average gene diversity differed by 6 and 13%, respectively, as compared with *s*=0.5. The outcome of the comparison between regions was not affected by the assumptions.

Table 6.4 Percentage of four classes of AFLP markers and number of unique markers in *Viola elatior*, *V. pumila*, and *V. stagnina* from the central (Dyje-floodplains, Czech Republic) and peripheral populations (Upper Rhine, Germany). P-values re from a χ2-test for differences between regions (d.f.=3).

			Classes of loci					
Species	**#markers**	**location**	**%cw**	**%rw**	**%cl**	**%rl**	***P***	**# unique markers**
V. elatior	152	Dyje	85.53	0.66	5.92	7.89	0.0178	1
		Rhine	86.84	5.26	1.32	6.58		6
V. pumila	155	Dyje	87.10	2.58	0.00	10.32	0.3533	2
		Rhine	85.81	3.23	1.94	9.03		1
V. stagnina	156	Dyje	75.64	4.49	0.64	19.23	0.0006	12
		Rhine	73.72	0.00	8.33	17.95		4

Classes were based on occurrence (marker found in ≥25% of populations per region: widespread, **w**; <25%: localised, **l**) and frequency of markers (average frequency ≥0.05: common, **c**; <0.05: rare, **r**). Classes: common widespread, **cw**; rare widespread, **rw**; common localised, **cl**; rare localised, **rl**.

Based on Mantel-tests between genetic and geographic distances among pairs of populations we rejected the null-hypothesis of regional equilibrium between gene flow and genetic drift for all species-region combinations. The scatterplots suggested that gene flow is relatively more important than genetic drift for the regional distribution of genetic variability in *Viola elatior* from the Upper Rhine, *V. pumila* from Rhine and Dyje, and *V. stagnina* from the Dyje-floodplains (Figure 1). This interpretation was supported by a comparison of variance in F_{st} among regions (Table 5). The variance ratio of resampled F_{st}-values between Rhine and Dyje was significantly lower than the observed variance ratio for *Viola elatior* (i.e. significantly stronger scatter of central populations) and significantly higher for *V. stagnina* and *V. pumila* (stronger scatter of peripheral populations). Average resampled F_{st}-values did not differ in *Viola elatior*, but population differentiation was significantly larger in peripheral populations and gene flow higher in central populations of the other two species.

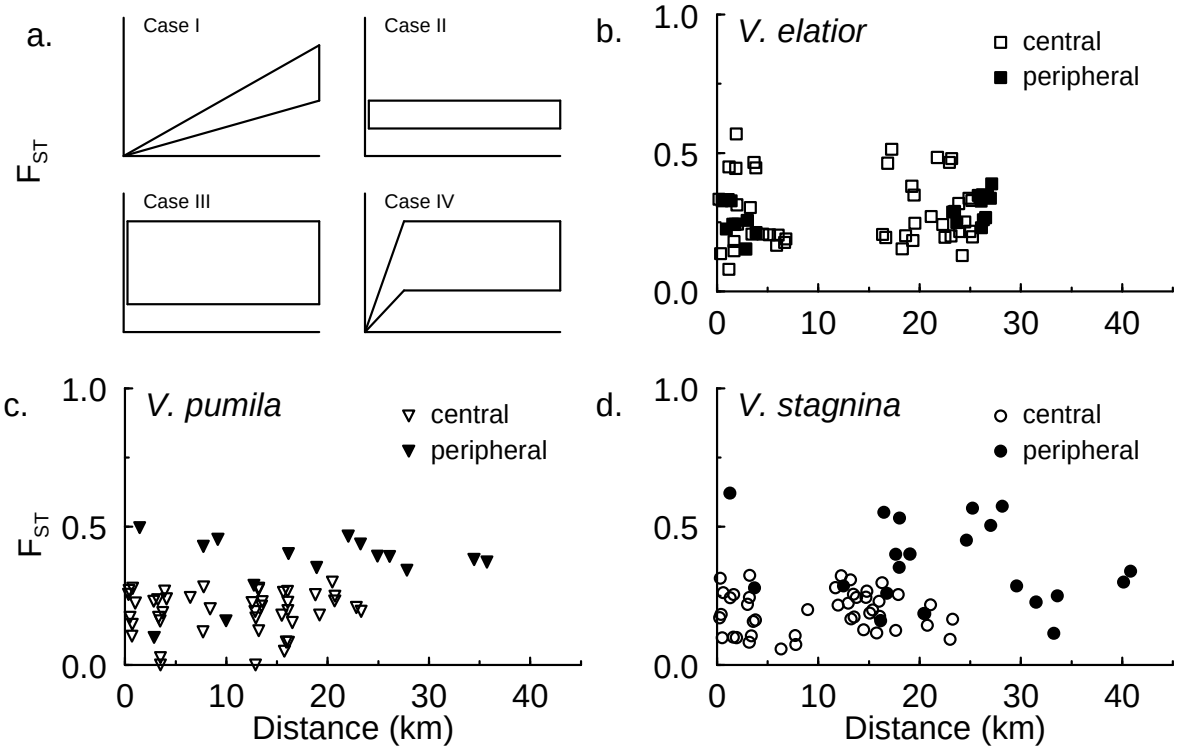

Figure 6.1 Theoretical (a.) and empirical relationships between genetic differentiation among pairs of populations (F_{ST}) and geographic distance (km) in *Viola elatior* (b.), *V. pumila* (c.), and *V. stagnina* (d.). Panel a. according to Hutchison & Templeton (1999), modified. Case I: equilibrium between genetic drift and gene flow, case II: non-equilibrium, gene flow relatively more important than genetic drift, case III: non-equilibrium, gene flow relatively less important than genetic drift, and case IV: lack of regional equilibrium, gene flow more important at shorter distances and drift more important at greater distances.

Within populations multivariate Mantel correlograms showed that there was significant spatial genetic structure up to about 10-20 m in populations of *Viola elatior* and *V. pumila* from both Rhine and Dyje (Fig. 2), and in *V. stagnina* from the Dyje floodplains. In peripheral populations of the latter species there was significant spatial genetic structure up to a distance of 10 m. Higher distance classes were only represented by very few individuals in the data set. For the first seven distance classes, where the Mantel correlograms revealed positive autocorrelation (i.e., 1-20 m), average genetic similarity was higher within peripheral populations than in core populations (Fig. 3). Differences between regions were significant in *Viola pumila* and *V. stagnina*.

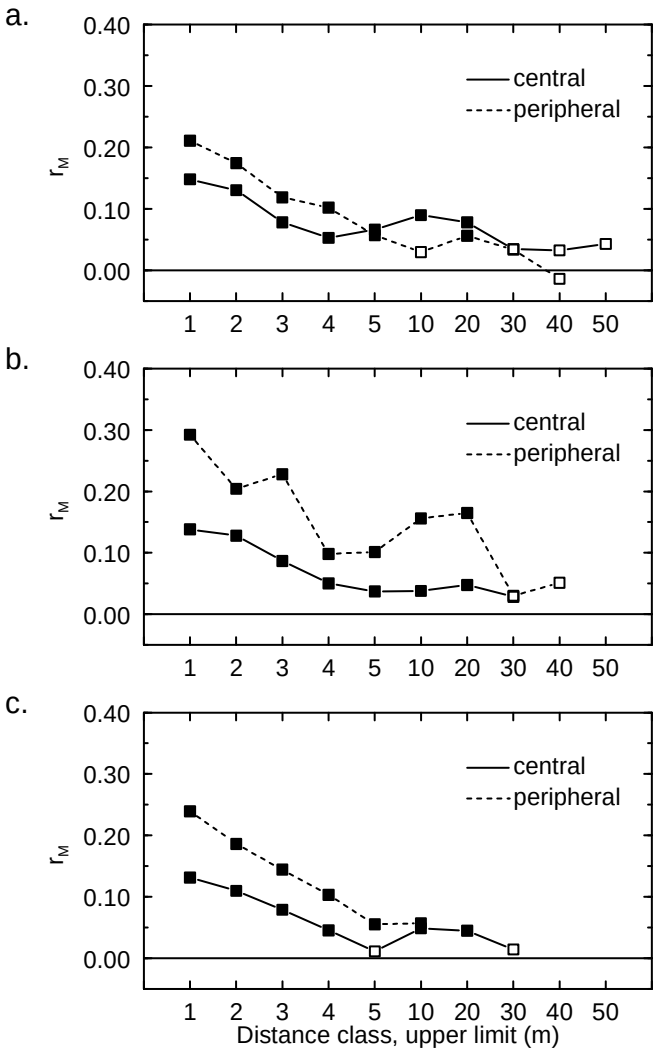

Figure 6.2 Multivariate Mantel correlogram showing spatial autocorrelation between genetic and geographic distance classes within populations of *Viola elatior* (a.), *V. pumila* (b.), and *V. stagnina* (c.). Filled symbols denote normalized Mantel statistics (r_M) that are significantly different from zero after progressive Bonferroni correction. Positive values of r_M show that individuals within a distance group are more genetically similar than pair of individuals at all other distances.

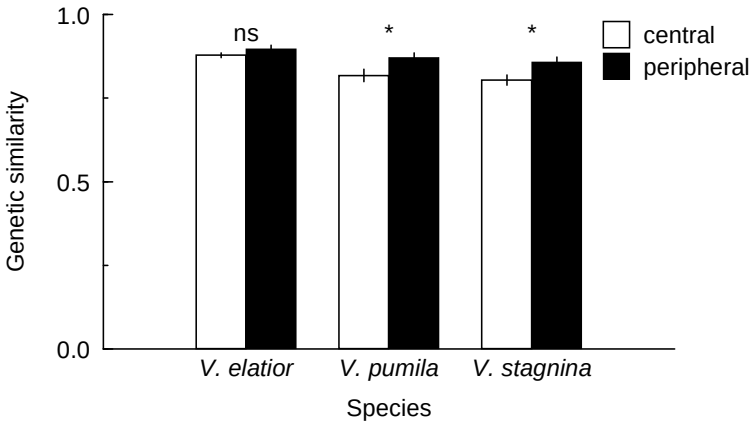

Figure 6.3 Average genetic similarity (Sørensen index) of pairs of individuals within populations of *Viola elatior*, *V. pumila* and *V. stagnina* in central (Dyje floodplains, Czech Republic) and peripheral populations (Upper Rhine, Germany) for distance classes from 0 to 20 m (mean ± s.e., *n*=7 classes, cf. Fig. 2). Asterisks indicate significant difference between groups at p<0.05 (permutation t-test, 10,000 permutations).

Table 6.5 Bootstrapped average F_{ST} for each species- region combination, ratio of the variance in F_{ST} between central (Dyje floodplains, Czech Republic) and peripheral populations (Upper Rhine, Germany). Bootstrapped 95% confidence limits in brackets.

Species	**Region**	**F_{ST} average**	**variance ratio**
V. elatior	Dyje	0.2817^{ns} (0.2469 – 0.3185)	0.2229^{ns‡}
	Rhine	0.2849 (0.2599 – 0.3089)	
V. pumila	Dyje	0.1916*** (0.1683 – 0.2129)	2.0362*
	Rhine	0.3637 (0.3064 – 0.4129)	
V. stagnina	Dyje	0.1924*** (0.1712 – 0.2134)	4.1957***
	Rhine	0.3635 (0.3014 – 0.4267)	

F_{st}-values were calculated with AFLP-SURV (Vekemans *et al.* 2002). Significantly higher average Fst and significantly higher variance in peripheral than in central populations indicate a stronger relative influence of genetic drift vs. gene flow on genetic structure (case III, cf. Fig. 1a).
Significance limits were obtained from Monte Carlo permutations, testing for the hypothesis that F_{st} (peripheral populations) > F_{st} (central populations), variance F_{st} (peripheral populations) > variance F_{st} (central populations). Bootstrap sample size was 10,000. Significance levels: ns, not significant (P>0.05); * (P<0.05); ** (P<0.01); *** (P<0.001).
‡ the variance ratio was significantly lower than the observed ratio, i.e. F_{st}-values show stronger scatter in central than in peripheral populations

Discussion

As predicted by the theory of range margins (Lawton 1993, Sagarin & Gaines 2002) population size (numbers of individuals) was consistently and significantly smaller in peripheral populations of the study species. Larger central populations have also been found in *Lychnis viscaria* (Lammi *et al.* 1999) and *Corrigiola litoralis* (Durka 1999), but size differences between peripheral and central populations were not evident in *Silene nutans* from Western Europe (Van Rossum 2003) and *S. regia* along an east-west gradient in North America (Dolan 1994). Contrary, adult *density* was higher in peripheral than in central populations of *Hornungia petraea* (Kluth & Bruelheide 2005). The same holds true for total adult density in permanent plots of the study species within central (25 adults m^{-2}) and peripheral (40 adults m^{-2}) populations (Eckstein & Otte, unpublished data), although densities of single life-cycle stages (except seedlings) did not differ significantly between regions owing to large within-group variation (Eckstein *et al.* 2004).

Isolation was larger in peripheral than in central populations of *Viola pumila* and *V. stagnina*, while the opposite relationships were found in *V. elatior*. It seems improbable that this was caused by differences in the intensity of population inventories between regions, since the study species are targets of intensive floristic inventories and monitoring programs in both regions (Eckstein *et al.* 2006a). Unlike the other two species, habitats of *Viola elatior* are prone to changes in species composition and environmental conditions in the course of forest succession. Analysis of herbarium collections suggests that there have been considerably more populations of this species present around the town of Lednice (CZ, northwest of Břeclav) during the 1950s (J.D., unpublished data). In response to habitat deterioration the species develops a 'remnant'-type of population dynamics (*sensu* Eriksson 1996), i.e. above-ground plant density slowly decreases as adult plants die until populations only persist as seeds in the soil. After major disturbance by logging the populations recur through germination from the seed bank (Eckstein *et al.* 2006a). The percentage cover of alluvial forests is much higher along the Dyje River than at the Upper Rhine, and in the former region many populations occurred in late-successional habitats within forest stands or along forest tracks. Therefore, we suggest that regional differences in availability of suitable, early- or mid-successional habitats may be responsible for the stronger isolation of population

in the core region. Fragmentation effects are hence superimposed on plant geographical patterns (core-periphery) in this species.

Peripheral populations of *Viola elatior* and core populations of *V. pumila* and *V. stagnina* were situated within an average distance of about 0.5 km to the nearest conspecific population, while central populations of *V. elatior* and peripheral populations of *V. pumila* were separated by about 1 km from their nearest neighbour. This is well within the average flight distance of bees and bumble bees (Hymenoptera, Apidae) for pollen and nectar, which is about 2.5 to 5 km (Eckert 1933, Araújo *et al.* 2004). However, since most bees feed at a distance of about 1 km or less around their colony (Eckert 1933), this distance may represent a limit for regular gene flow through pollen (cf. Kwak *et al.* 1998 and references therein). This is supported by our analysis of the *relative* importance of gene flow and genetic drift. There was no equilibrium between these processes in the study species. The relationships between genetic and geographic distances suggested a stronger effect of gene flow for all species-region combinations with an average distance to the nearest conspecific population of about 0.5 km or less. The influence of drift was larger than that of gene flow for those species-region combinations separated by about 1 km and especially in peripheral populations of *V. stagnina* that were situated, on average, 5 km apart from the nearest population. Stronger genetic divergence among peripheral populations of *Viola pumila* and *V. stagnina* (F_{st}-values) further supported this view. Similarly, Culley and Grubb (2003) found non-equilibrium conditions and a very similar scatter between genetic and geographic distance than in the present study, indicating that in fragmented populations of *Viola pubescens* (pairwise distances ranged from 0.3 to 45 km) genetic drift had much stronger influence than gene flow on genetic population structure.

Divergence among populations tended to be higher in peripheral than in central populations of *Silene nutans* (Van Rossum *et al.* 2003). Similar differentiation between populations as in the present study has been reported from other cleistogamous (Auge *et al.* 2001, Culley & Grubb 2003), selfing (Durka 1999), or rare plant species (Travis *et al.* 1996, Lammi *et al.* 1999, Schmidt & Jensen 2000). Genetic divergence among populations was high in three rare species of the genera *Silene* and *Lychnis* (Dolan 1994, Lammi *et al.* 1999, Van Rossum *et al.* 2003), whereas gene flow was still high in the common *Lychnis flos-cuculi* (Galeuchet *et al.* 2005). In a study on eight populations of *Viola elatior* from Austria, Germany, Italy, and Switzerland, 82% of the genetic variance rested among

populations, while the remaining 18% were found within populations (Gygax 2001). The differences to the present study are most probably due to the much larger geographic range sampled in that study which results in a larger among population genetic differences.

Assuming constant ecological niches over time, the species have most probably been more widely distributed and more frequent at the end of the last glaciation when climatic conditions in Central Europe were more continental (Younger Dryas period, 12000 year BP; Frenzel 1968, Burkart 2001). With the development of the current climate and the increase of human influence on the landscape, they retreated to floodplains, which provide regionally sub-continental conditions, flood disturbances, and only low to moderate human land use. The analysis of the past distribution suggests that all three species have undergone a severe decline during the last decades (Eckstein *et al.* 2006a), which caused strong fragmentation and isolation of populations. Non-equilibrium metapopulations with few or no recolonizations provide ideal conditions for population divergence (Harrison & Hastings 1996), which is supported by the present data. The large proportion of genetic variance still present within populations may be a result of the perennial nature of the species and/or the presence of a persistent seed bank. The conservation of genotypes through time in a soil seed bank reduces genetic divergence among populations and may increase genetic diversity (Cabin *et al.* 1998, McCue & Holtsford 1998, Morris *et al.* 2002). Therefore, populations of perennials with a persistent seed bank may be a patchwork of genotypes from a spatial as well as from a temporal point of view.

Higher F_{st}-values and higher average similarity within peripheral populations indicated that gene flow at the local scale may be higher in peripheral than in central populations for two of the three species. This may be owing to differences in agricultural management between regions (Eckstein *et al.* 2004). Peripheral populations are situated exclusively in nature conservation areas or managed under conservation contracts, whereas populations in the Czech Republic are found mainly in irregularly managed sites. Regular management improves stand and population stage structure of the species and increases densities of seedlings (Eckstein *et al.* 2004) and total adult plants, as well as fecundity in peripheral populations (Eckstein *et al.*, unpublished data). Higher densities of mature (flowering) plants are linked to shorter pollinator flight distances but higher percentage interplant flights in *Viola* (Beattie 1976). The latter enhances

interplant gene exchange, and percent cross pollination. Leptokurtic distributions of pollinator flight distances will restrict gene flow and enhance the divergence of sub-populations at a very localised scale (Beattie 1976). In contrast, in wind-pollinated Sitka spruce strong spatial genetic structure in peripheral but not core populations was probably caused by overlapping seed shadows owing to higher density of adults in core populations (Gapare & Aitken 2005).

Absence of rare localised genetic markers in peripheral populations demonstrates possible effects of genetic drift. Absence of some widespread markers in peripheral populations of the most isolated species (*V. stagnina*) may even indicate genetic erosion. However, the existence of *V. stagnina* var. *lactaeoides* in the Netherlands (Weeda 2001), though with uncertain taxonomic status, suggests that isolation and genetic divergence from the original taxon may promote speciation at the boundaries of the species range (Lesica & Allendorf 1995). Owing to post-glacial range contraction and recent habitat fragmentation there is no equilibrium between gene flow and genetic drift in the study species. Despite this, the balance between gene flow and genetic drift still shapes genetic diversity. In metapopulations of these insect-pollinated plants with an average distance of <1 km to the nearest neighbour, gene flow is sufficiently large to outweigh the effects of random genetic drift and retain relatively high levels of genetic similarity between populations. Larger average distances to the nearest population, as is often the case at the margin of species ranges, will reduce gene flow to levels that are no longer capable to counterbalance genetic drift. Consequently, random loss of alleles will lead to increasing genetic differentiation between populations and loss of regional genetic diversity. However, colonisation history and responses to habitat fragmentation are species-specific. We suggest that conservation of the floodplain violets should include peripheral populations since they contained a number markers lacking in central populations and thus contributed to genetic diversity at the species-level (cf. Safriel *et al.* 1994, Lesica & Allendorf 1995, Durka 1999).

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Appendix 1. Geographic location of the study populations (northern latitude (N) and eastern longitude (E)) and number of sampled individuals (*n*) in the Czech Republic (Morava-Dyje floodplains) and in Germany (Upper Rhine).

	Czech Republic				Germany			
Species	**ID**	**N**	**E**	***n***	**ID**	**N**	**E**	***n***
Viola elatior	EC1	48°49′24′′	16°46′27′′	20	ED1	49°35′59′′	08°25′44′′	19
	EC2	48°48′43′′	16°49′05′′	19	ED2	49°35′54′′	08°26′53′′	16
	EC3	48°49′25′′	16°46′36′′	11	ED3	49°49′51′′	08°24′18′′	20
	EC4	48°49′16′′	16°49′35′′	8	ED4	49°50′03′′	08°25′34′′	20
	EC5	48°49′33′′	16°48′06′′	14	ED5	49°50′20′′	08°24′09′′	20
	EC6	48°37′58′′	16°57′11′′	20	ED6	49°48′31′′	08°25′42′′	20
	EC7	48°41′33′′	16°56′50′′	16	ED7	49°35'46"	08°25'59"	20
	EC8	48°41′04′′	16°56′13′′	20				
	EC9	48°37′55′′	16°57′28′′	20				
	EC10	48°38′49′′	16°57′39′′	20				
			Σ	**168**			**Σ**	**135**
Viola pumila	PC1	48°48′43′′	16°49′05′′	20	PD1	49°36′03′′	08°27′01′′	20
	PC2	48°48′43′′	16°49′39′′	20	PD2	49°36′41′′	08°26′19′′	20
	PC3	48°49′24′′	16°46′27′′	19	PD3	49°40′05′′	08°22′37′′	19
	PC4	48°49′08′′	16°46′31′′	20	PD4	49°48′33′′	08°25′43′′	20
	PC5	48°48′47′′	16°49′20′′	20	PD5	49°50′06′′	08°25′36′′	18
	PC6	48°43′01′′	16°55′08′′	20	PD6	49°55′05′′	08°22′30′′	18
	PC7	48°41′53′′	16°57′14′′	20				
	PC8	48°42′36′′	16°54′36′′	20				
	PC9	48°42′38′′	16°55′13′′	20				
	PC10	48°38′31′′	16°55′57′′	20				
			Σ	**199**			**Σ**	**115**
Viola stagnina	SC1	48°48′44′′	16°49′39′′	19	SD1	49°51′33′′	08°23′29′′	18
	SC2	48°49′26′′	16°46′44′′	18	SD2	50°01′16′′	08°54′09′′	18
	SC3	48°48′47′′	16°49′20′′	20	SD3	49°53′03′′	08°49′43′′	18
	SC4	48°49′20′′	16°46′52′′	20	SD4	49°59′28′′	08°31′40′′	20
	SC5	48°48′57′′	16°49′16′′	19	SD5	50°03′29′′	08°44′16′′	20
	SC6	48°43′09′′	16°54′04′′	20	SD6	49°59′05′′	08°30′47′′	15
	SC7	48°42′37′′	16°55′08′′	19	SD7	49°50′01′′	08°25′27′′	11
	SC8	48°42′36′′	16°54′43′′	20				
	SC9	48°38′30′′	16°65′01′′	20				
	SC10	48°41′54′′	16°55′52′′	18				
			Σ	**193**			**Σ**	**120**
			Total	**560**			**Total**	**370**

Chapter 7

Extended summary and conclusions.

In the following the results of the studies compiled in the present work are summarised and discussed in the light of the main aims presented in **Chapter 1** (p. 6). For detailed results of individual papers, Figures and Tables the reader is referred to the respective chapters 2-6.

A. Influence of stochastic and deterministic processes on population viability

‹Chapters 2, 3, 4, 6›

In **Chapter 2** single seedlings of *V. elatior*, *V. pumila* and *V. stagnina* that germinated in trays under identical conditions were transplanted into experimental grassland plots of 0.1 m^2 size in a common garden. Plots were subject to one of the following four manipulations of interspecific interactions (C, clipping) and water availability (W, water addition):

- C+W+, surrounding vegetation clipped, 1 L of water added every second day,
- C+W-, surrounding vegetation clipped, no extra water,
- C-W+, not clipped, 1L of water added every second day, and
- C-W-, not clipped, no extra water.

In the C+ treatments aboveground diffuse competition by the surrounding grassland vegetation was consistently reduced since plots were mown repeatedly throughout the experiment and water availability was either increased or natural. In the C- treatments, seedlings experienced competition by other species and received either extra water or not. Plots were located in three grassland types that owing to their position and soil type represented a natural soil moisture gradient. The experiment thus consisted of a factorial combination of three grassland types (dry, mesic, wet), three species (*V. elatior*, *V. pumila* and *V. stagnina*), two clipping treatments and two watering treatments with five replicates per treatment combination.

The fate of seedlings (alive, dead) was censored during ten occasions from 19 May to 23 September. Additionally, height, number of leaves and numbers of capsules (cleistogamous reproduction) of each plant was recorded. These data served to analyse separately the effects of competition and environmental variation (i.e. *environmental stochasticity*) on survival, growth and reproduction.

Results of this experiment showed that these vital rates were differently affected by interspecific interactions and water availability. Survival analysis (Fox 1993) revealed that cumulative seedling survival was significantly higher in wet grassland than in the other grassland types and that survival on plots receiving extra water was significantly higher than on nonwatered plots. Thus water availability had the strongest influence on the survival of seedlings. The crucial role of sufficient available water for seedling germination and survival has also been shown by, e.g., De Jong and Klinkhamer (1988) and Xiong *et al.* (2003). In two biennial plant species of sand dune systems seedling mortality was significantly correlated with soil water content and increased strongly below 5% soil d.wt. (De Jong & Klinkhamer 1988), as in the present study (**Chapter 2**).

Grassland type, water addition and species effects accounted for most of the variation in height growth rate (HGR), whereas leaf accumulation rate (LGR) and reproduction were affected by grassland type, clipping and water addition. Water availability was not sufficient for production of capsules: in the dry and mesic grassland, plants receiving additional water only produced capsules on clipped plots. In the wet grassland, capsule production was higher on clipped than on unmown plots.

In **Chapter 3** population stage structure of the study species was investigated in a total of 122 plots of 0.1 m^2 area each in 27 field populations from two regions (Upper Rhine, Germany; March-Dyje floodplains, Czech Republic). Individual plants were marked in 2001, classified into five stage classes according to the presence of flowers and number of shoots, i.e. (1) seedlings, (2) small vegetative plants, (3) large vegetative plants, (4) small flowering, and (5) large flowering plants (cf. **Chapter 3**, *Material and Methods*, *Life-cycle stages*) and revisited in 2002. Using analysis of variance (ANOVA; Quinn & Keough 2002) we studied the effects of species, region (for differences between central and marginal populations, see below) and year (i.e. *environmental stochasticity*) on stage *density*. Additionally, the effects of year and management and year and population (i.e. spatial variation) on stage *frequency* were analysed using two log-linear models (Quinn & Keough 2002).

Seedling density varied significantly between regions and between years (**Chapter 3**), whereas there were only species specific differences in density of small and large vegetative plants. In all three species the frequency of life-cycle stages, i.e. population stage structure (Oostermeijer *et al.* 1994a), was significantly affected by the factors year, i.e. *environmental stochasticity*, and region, which could be attributed to *habitat quality*. A direct comparison of year and management (regular *vs.* irregular) showed that in *V. elatior* and *V. stagnina* both factors had similar and significant effects on population stage structure, whereas in *V. pumila* stage frequency differed only between years (**Chapter 3**).

Since all populations at the Upper Rhine were situated exclusively in nature conservation areas or are managed under conservation contracts, while the Dyje populations were found mainly in irregularly managed meadows, the effects of management and region could not be easily separated. However, after grouping populations either according to region (Rhine *vs.* Dyje) or according to management (regular *vs.* irregular) the number of seedlings varied significantly more between managed and unmanaged populations than between the two regions in *V. elatior* and *V. stagnina*. In *V. pumila* differences were small.

These findings were confirmed by the analyses presented in **Chapter 4** based on the demography of the three species. Data on the fate of marked plants in permanent plots of 27 populations from the two regions were used to construct life-cycles of the study species. We pooled the information of three to six populations per species-region combination to obtain robust 5×5 Levkovitch transition matrices, based on five life-cycle stages. Using matrix models (cf. Caswell 2001) we obtained observed and bootstrapped population growth rates (λ_O and λ_B, respectively), observed and stable stage distributions, net reproductive rate (R0), generation time, and age at first reproduction.

Observed population growth rates of all species-region combinations except for *V. stagnina* at the Rhine were <1 but λ_B did not differ significantly between species or regions. Since λ is influenced by climatic conditions this indicated that conditions did not differ substantially (at least with respect to their effect on λ) between regions. A long-term λ of around 1 can be expected in perennials as long as the populations do not go extinct (e.g., Solbrig *et al.* 1980; Newell *et al.* 1981; Silvertown *et al.* 1993; Svensson *et al.* 1993; Oostermeijer *et al.* 1996; Nicolè *et al.* 2005).

Despite higher probabilities of survival and reproduction of plants in Dyje populations, R0 was similar or higher in populations at the Upper Rhine. We suggest that this may be an effect of differences in habitat quality owing to management and herbivory. In Dyje populations a considerable but variable proportion of capsules was infested by larvae of *Orbitis cyaneus* (L.), a beetle of the Curculionidae family, while this phenomenon has not been observed in the Rhine populations. Data from marked plants suggest that insects can substantially reduce seed production. Additionally, a lack of regular management through mowing leads to litter accumulation and bryophyte encroachment in Dyje populations. Thick litter layers on which seeds are shed will hamper seed germination and seedling establishment (Facelli & Pickett 1991 and references therein), which contrasts with facilitative effects of small amounts of litter on top of seeds (Eckstein & Donath 2005).

Analysis of population genetic structure and the importance of genetic drift, i.e. *genetic stochasticity*, of 50 populations of the study species in **Chapter 6** using AFLP analysis (cf. Mueller & Wolfenbarger 1999), showed that genetic diversity was lower and the *relative importance* of genetic drift higher than that of gene flow in Rhine populations of *V. pumila* and *V. stagnina*. However, none of the species-region combinations was in equilibrium with respect to genetic drift and gene flow and genetic diversity was not correlated with population size. A significant but rather small proportion of the total genetic variation of each species (about 6%) was found between regions. Consistently higher values of λ of Rhine populations despite lower genetic diversity showed that genetic stochasticity did not lead to lower population viability of these populations. We suggest that this may be a consequence of regular management that improves habitat quality of Rhine populations.

Deterministic processes such as human land-use and management regimes, habitat loss, fragmentation, and succession act at the landscape scale (**Chapter 1**, cf. Fig. 1.1) and often pertain to whole populations. In contrast, stochastic processes affect individuals within local populations. Unpredictable variation in environmental conditions will cause the mean values of survival, growth and reproduction across all individuals to vary from year to year. Environmental stochasticity and its extremes, i.e. extremely bad (*catastrophes*) and extremely good years (*bonanzas*), may result in strong correlation between vital rates, since good years for survival are usually also good years for growth and *vice versa* (Caswell 2000; Morris & Doak 2002). In contrast, demographic and genetic

stochasticity will affect single individuals within the population independent of their life-cycle stage. Therefore, the magnitude of change caused by environmental stochasticity will largely be unaffected by population size, whereas the effects of demographic and genetic stochasticity depend on the number of individuals within a population. Since random variation in survival and reproduction among individuals is compensated in large populations, demographic and genetic stochasticity will be a larger problem in populations of small size.

Intensification and abandonment of land use are both associated with (i) an increasing rate of fragmentation of habitats and (ii) changes in habitat quality. Fragmentation will increase the extinction risk of local populations as an effect of reduced population sizes and increased edge effects and decrease the rate of colonisation owing to increased isolation of local populations (Hanski 1999; Debinski & Holt 2000; Saunders *et al.* 2001; Eriksson *et al.* 2002). Assuming constant ecological niches over time, the study species have most probably been more widely distributed and more frequent at the end of the last glaciation when climatic conditions in Central Europe were more continental (Younger Dryas period, 12000 year BP; Firbas 1949; Frenzel 1968). With the development of the current climate and the increase of human influence on the landscape, they retreated to floodplains, which provide regionally sub-continental conditions, flood disturbances, and only low to moderate human land use. The analysis of the past distribution suggests that all three species have undergone a severe decline during the last decades (Eckstein *et al.* 2006a). As 'new rares' (*sensu* Oostermeijer 1996) the study species are therefore subject to recent fragmentation and isolation of local populations.

Both common and rare plant species show lower density and individual fitness (Widén 1993; Kéry *et al.* 2000; Hooftman *et al.* 2003; Lienert *et al.* 2002; Lienert & Fischer 2003; Vergeer *et al.* 2003a, b) in response to fragmentation. Density, stage structure, seed set and damage through herbivory in the common wetland specialist *Primula farinosa* were affected by either of the three aspects of fragmentation, i.e. reduced population size, increased isolation and edge effects (Lienert & Fischer 2003). The response to fragmentation is species-specific (Fischer & Stöcklin 1997; Hooftman *et al.* 2003; Lindborg *et al.* 2005) and depends on species traits (Honnay *et al.* 2005), i.e. clonal species or those with few and/or heavy diaspores, lack of dispersal structures will be more negatively affected by isolation than species with better dispersal ability (Kolb & Diekmann 2005).

However, within the cultural landscape, fragmentation is the endpoint and consequence of a development that is related to other deterministic processes, which may erase local populations and threaten species survival. Most of these processes slowly reduce habitat quality, which may also lead to decreasing population sizes (e.g., Eisto *et al.* 2000; Endels *et al.* 2002). Small populations, in turn, are more prone to extinction than large ones (e.g., Fischer & Stöcklin 1997; Matthies *et al.* 2004).

An analysis of the German red list of threatened vascular plants showed that 66% of 819 extinct and endangered species are threatened by habitat destruction, mostly civil works such as building of roads and other infrastructure and the growth of villages, towns, and industrial plants (Korneck *et al.* 1998). The second most important factor threatening 56% of the species are agricultural land use practices. Interestingly, both intensification and abandonment of land use affect similar numbers of species. Habitat changes, e.g., through the input of nutrients (Lee & Caporn 1998; Aerts & Bobbink 1999) and by reducing natural habitat dynamics threaten 49% of the species. The latter processes have become more important during recent years (Korneck *et al.* 1998).

Processes responsible for the decline of the study species are (i) grassland transformation into crop fields after 1945, grassland eutrophication, abandonment and afforestation of meadows, (ii) lowering of the ground water table and drainage, (iii) removal of small-scale habitat elements (e.g., fringes, hedgerows, ditches), (iv) intensive grazing, (v) peat and sand mining and soil filling, and (vi) reduced flood dynamics (Korneck *et al.* 1998).

Large effects of management on populations stage structure (**Chapter 3**), density of seedlings (**Chapter 3**), net reproductive rate (**Chapter 4**) and large variation between regions with different management practice (**Chapters 3, 4, 6**) suggest that deterministic processes present the strongest threat for the viability and persistence of populations of the three floodplain violets. Deterioration of habitat quality owing to reduced management will lead to population growth rates <1, high mortality of seedlings, reduced flowering and seed set (Jensen & Meyer 2001; Jacquemyn *et al.* 2003; Brys *et al.* 2005). Besides these, environmental stochasticity (soil moisture, interspecific interactions, and climatic conditions) is also important especially for seedling survival and reproduction (**Chapters 2, 3**). In some years environmental stochasticity (e.g., climatic conditions) may have an overriding effect over other factors such as population size (cf. Morgan 1999). Genetic stochasticity (i.e. genetic drift) is active and important in

all species-region combinations (**Chapter 6**) and seems to have a differential influence on small *vs*. large or nearby *vs*. isolated populations of the study species.

Deterministic processes comprise reduced management of the non-intensively used sub-continental floodplain meadows, the succession from open forest fringes and paths to closed mature alluvial forests (concerning especially *V. elatior*), habitat destruction, and reduced disturbance through flooding. Habitat quality is decreased through litter accumulation, bryophyte encroachment and colonization by shrubs and trees. Through suitable management such as mowing, viable populations can be conserved even at the western margin of the species ranges along the Upper Rhine in Germany (**Chapters 3, 4**). However, since the study species are perennial it remains open whether these populations are safe or whether they represent remnant populations (Eriksson 1996). It is not clear for the study species whether habitat destruction and fragmentation has proceeded so far as to induce an extinction debt (Tilman *et al.* 1994; Honnay *et al.* 2005). On the other hand, successful efforts are undertaken to actively establish new populations of rare floodplain species, including the study species, by the transfer of hay from species-rich meadows to abandoned crop fields and grasslands (Donath *et al.* 2003, 2004, 2006; Hölzel & Otte 2003).

Conclusions (1)

- **Deterministic processes, such as habitat destruction, decreased habitat quality, and succession seem to play a major role for population viability and persistence of the study species.**
- **Additionally, environmental stochasticity will affect population stage structure and seedling recruitment.**
- **Negative effects of fragmentation (isolation, small population size, edge effects) can be balanced by suitable conservation management in the study species.**
- **It remains an open question, whether extant populations of these perennials are also viable in the long-term or represent remnant populations prone to extinction due to an extinction debt.**

B. Differences in demography and genetic structure between central and marginal populations and the importance of genetic drift and gene flow

‹*Chapters 3, 4, 6*›

The abundance and density of individuals and population frequency are not constant across a species range but usually decrease towards the range margin (Lawton 1993; Lesica & Allendorf 1995; '*abundant centre hypothesis*', cf. Sagarin & Gaines 2002). Although patterns and ecological processes at the range margin have received attention from plant ecologists (e.g. Carter & Prince 1981; Bengtsson 1993; Lennon *et al.* 1997; Nantel & Gagnon 1999; Kluth & Bruelheide 2005), conservationists and plant geneticists (Safriel *et al.* 1994; Lesica & Allendorf 1995 and references therein; Durka 1999; Lammi *et al.* 1999; Lönn & Prentice 2002; Van Rossum *et al.* 2003), explicit tests of the abundant centre hypothesis have been ambivalent (Sagarin & Gaines 2002 and references therein).

Decreasing abundance and frequency of populations from the range centre to the margin may be an effect of low habitat quality at the range margin affecting species performance and reducing reproduction and dispersal (Pigott & Huntley 1981; García *et al.* 2000; Dorken & Eckert 2001). Additionally, the habitat type or safe-sites for germination may be infrequent at the range margin (Dinsdale *et al.* 2000; Jump & Woodward 2003). Therefore, peripheral plant populations will often be (i) more isolated (Lawton 1993; Lesica & Allendorf 1995) and (ii) contain fewer individuals than central populations (Durka 1999; Lammi *et al.* 1999; but see Kluth & Bruelheide 2005). Small populations face an increased risk of extinction through environmental stochasticity or catastrophes (Lande 1993; Menges & Dolan 1998), and the viability of these populations may also be reduced because of the increased chance of mating between relatives (Menges 1991; Fischer & Matthies 1998). Additionally, small and/or isolated populations may suffer from pollinator limitation (Jennertsen 1988; Ågren 1996), which may further reduce seed production (Morgan 1999). Low habitat quality and/or small population size at the range margin may lead to increased variability of demographic rates (Nantel & Gagnon 1999) and, consequently, to higher rates of extinction, while larger distances between suitable habitats and source populations may reduce the rate of re-colonisation of empty habitats, lowering the overall proportion of occupied patches.

The three study species share a continental distribution with a centre of occurrence in the temperate zone of Eastern Europe and Western Siberia, and reach their western

range margin in Central and Western Europe (Eckstein *et al.* 2006a). Populations along the Upper Rhine (Germany) therefore belong to the species range margin, whereas populations in the March-Dyje floodplains (Czech Republic) are close to the centre of distribution.

In **Chapters 3** and **4**, we compared population stage structure and demography between marginal and central populations based on data from permanent plots. In contrast to expectations, seedling density (**Chapter 3**) and total density were higher in marginal than in central populations. This was also found by Kluth and Bruelheide (2005) for *Hornungia petraea*, whereas the abundant centre hypothesis was confirmed for *Cirsium heterophyllum* (Jump & Woodward 2003). Lower densities in the latter species were related to lower fecundity at the range margin, which has also been observed for other species (e.g., Pigott & Huntley 1981; García *et al.* 2000; Dorken & Eckert 2001). In *Hornungia petraea* differences in fecundity between regions were small (Kluth & Bruelheide 2005) and differences in density are probably related to seed bank dynamics. In the study species, the probabilities of survival and reproduction were higher in central populations but fecundity and net reproductive rate were higher (or similar) in marginal populations than in central ones (**Chapter 4**). This was most probably an effect of population management through mowing (see above), which outweighed factors responsible for the presumed plant geographical patterns. Population growth rates (λ) were higher in marginal populations but differences were not statistically significant (**Chapter 4**).

In accordance to the abundant centre hypothesis, marginal populations were smaller (lower numbers of individuals) in all three species and stronger isolated in *V. pumila* and *V. stagnina* (**Chapter 6**). In case of *V. elatior*, central populations were more isolated than marginal ones. The latter was most probably an effect of a higher percentage cover of alluvial forests along the Dyje River than at the Upper Rhine. In the former region many populations occurred in late-successional habitats within forest stands or along forest tracks. Therefore, regional differences in availability of suitable, early- or mid-successional habitats may be responsible for the stronger isolation of populations in the core region. Fragmentation effects were hence superimposed on plant geography (core-periphery) in *V. elatior* (**Chapter 6**).

Similarly, population genetic analysis revealed that genetic diversity, measured as gene diversity (Lynch & Milligan 1994) and molecular variance (Fischer & Matthies 1998),

number of markers (=scorable bands) or percentage of polymorphic markers (=bands) was lower in marginal populations of *V. pumila* and *V. stagnina*, whereas there were no significant differences in *V. elatior* (**Chapter 6**).

Peripheral populations of *Viola elatior* and core populations of *V. pumila* and *V. stagnina* were on average about 0.5 km away from the nearest conspecific population, while central populations of *V. elatior* and marginal populations of *V. pumila* were separated by about 1 km. This is within the average flight distance of bees and bumble bees (Hymenoptera, Apidae) for pollen and nectar, which is about 2.5 to 5 km (Eckert 1933, Araújo *et al.* 2004). Since most bees feed at a distance of about 1 km or less around their colony (Eckert 1933), this distance may represent a limit for regular gene flow through pollen (cf. Kwak *et al.* 1998 and references therein). This is supported by our analysis of the relative importance of gene flow and genetic drift (**Chapter 6**).

Based on a stepping-stone model of population structure, i.e. a model in which gene flow is most likely between neighbouring populations, Hutchison and Templeton (1999) proposed an approach to analyse the relative importance of gene flow and genetic drift through the relationships between genetic (F_{ST}) and geographic distances (see **Chapter 6**). A pattern consistent with equilibrium between gene flow and drift, i.e. isolation by distance, should be characterised by a positive monotonic relationship between genetic and geographic distance. Because of the homogenising effect of gene flow, populations at closer distances should not only be separated by smaller genetic distance but also the variation in genetic distance should be lower. As geographic distances increase the relative effect of gene flow decreases and widely separated populations are both genetically more distant and genetic distance shows larger variation due to genetic stochasticity. However, if a region has not been occupied by a species for long enough or if dispersal is limited, no equilibrium between gene flow and drift will have developed.

In such cases missing correlation between genetic and geographic distances with little variation in F_{ST} would indicate higher relative importance of gene flow over genetic drift (Hutchison & Templeton 1999). If gene flow remains relatively strong in comparison with random genetic drift this patterns will persist. However, if environmental conditions lead to fragmentation and isolation of populations within the region genetic drift will become more influential and there will be large variation in F_{ST}.

Based on the approach of Hutchison and Templeton (1999), there was no equilibrium between gene flow and genetic drift in the study species. The relationships between genetic and geographic distances suggested a stronger effect of gene flow for all species-region combinations with an average isolation distance of about 0.5 km or less. The influence of drift was larger than that of gene flow for those species-region combinations separated by about 1 km and especially in peripheral populations of *V. stagnina* that were situated on average 5 km apart. Stronger genetic divergence among marginal populations of *V. pumila* and *V. stagnina* (Φ_{ST}-values) further supported this view.

Similarly, Culley and Grubb (2003) found that in fragmented populations of *Viola pubescens* (pairwise distances ranged from 0.3 to 45 km) genetic drift had much stronger influence than gene flow did on genetic population structure. Divergence among populations tended to be higher in peripheral than in central populations of *Silene nutans* (Van Rossum *et al.* 2003). Similar differentiation between populations as in **Chapter 6** has been reported from other cleistogamous (Auge *et al.* 2001; Culley & Grubb 2003), selfing (Durka 1999), or rare plant species (Travis *et al.* 1996; Lammi *et al.* 1999; Schmidt & Jensen 2000). Genetic divergence among populations was high in three rare species of the genera *Silene* (Dolan 1994; Lammi *et al.* 1999; Van Rossum *et al.* 2003), whereas gene flow was still high in the common *Silene flos-cuculi* (Galeuchet *et al.* 2005).

Unlike in many other studies (e.g., Dolan 1994; Travis *et al.* 1996; Fischer & Matthies 1998; Lammi *et al.* 1999; Culley & Grubb 2003) genetic diversity was not significantly correlated with population size or isolation in the study species. Relationships between actual population size and molecular variance can be masked by population history (age, founder effects), the spatial configuration (isolation, number of and distance to other populations) or chance events (Oostermeijer *et al.* 1994b; Schmidt & Jensen 2000).

Marginal populations of *V. elatior*, *V. pumila*, and *V. stagnina* lost one, two, and eight rare localised loci, respectively. Additionally, peripheral populations of *Viola stagnina* lacked one rare widespread locus and three common widespread loci that occurred in ≥40% of the core populations and had an average frequency of ≥0.1120. Loss of rare localised genetic markers in marginal populations further demonstrated the effects of random genetic drift, especially in *V. stagnina*.

At the same time, marginal populations already contained a few new loci lacking in central populations. Peripheral populations contained one unique common widespread locus in *V. elatior*, one in *V. pumila*, and one common localised locus in *V. stagnina*. This

genetic novelty increases the total genetic variation of the species making these peripheral populations potentially valuable for conservation (Safriel *et al.* 1994; Lesica & Allendorf 1995; Durka 1999). The existence of *V. stagnina* var. *lactaeoides* in the Netherlands (Weeda 2001), though with uncertain taxonomic status, suggests that isolation and genetic divergence from the original taxon may promote speciation at the outer boundaries of the species range (Lesica & Allendorf 1995).

C o n c l u s i o n s (2)

- **Predictions of the abundant centre hypothesis could only partly be verified for the study species: population sizes were consistently smaller in marginal populations than in central ones. In contrast, the densities of seedling and total densities were higher in marginal populations.**
- **Stronger isolation and lower genetic diversity were found in marginal populations of *V. pumila* and *V. stagnina*.**
- **In case of *V. elatior* fragmentation effects due to different landscape structures in marginal and central populations were superimposed on plant geography, leading to weaker isolation of marginal populations. Genetic diversity did not differ between regions.**
- **Gene flow had a stronger relative influence on genetic structure than genetic drift (i.e. *genetic stochasticity*) in all species-region combinations with average distances <0.5 km, whereas genetic drift was more important, if average distances were >1 km.**
- **There was no equilibrium between gene flow and drift in the study species and genetic diversity was not significantly correlated with population size or isolation.**

C. The effects of mating system and pollen source on seed production and offspring performance

‹Chapter 5›

Many plant species that have been more widespread in the past, are today restricted to small and isolated populations as a result of land use changes, habitat destruction and fragmentation (e.g., Saunders *et al.* 1991; Jensen & Schrautzer 1999; Lienert *et al.* 2002; Eriksson *et al.* 2002; Honnay *et al.* 2004). Small and isolated populations meet an increased risk of extinction through environmental stochasticity or catastrophes (Lande 1993; Fischer & Stöcklin 1997; Matthies *et al.* 2004), but viability of these populations may also be reduced because of the increased chance of mating between relatives in small populations. Pollen may originate from anthers of the same flower (autogamous selfing), from a different flower on the same shoot or genet (geitonogamous selfing), or a shoot from another genet (xenogamy, crossing) (Richards 1997). Thus, especially in small and/or isolated populations there is a large probability for self pollination (Barrett & Kohn 1991; Dudash & Fenster 2000).

Selfing may lead to inbreeding depression (e.g., Husband & Schemske 1996; Culley 2000; Dudash & Fenster 2000; Charlesworth 2003), i.e. the reduction in viability and fitness (e.g., Waller 1984; Mitchell-Olds & Waller 1985; Dudash 1990) in selfed as compared to outcrossed progeny, leading to increased abortion of ovules, and reduction in seed set, germination, offspring fitness and population genetic structure (Waller 1984; Dudash 1990; Fischer & Matthies 1997; Richards 1997; Berg & Redbo-Torstensson 1999; Culley 2000; Karrenberg & Jensen 2000; Lienert & Fischer 2002).

While most open self-compatible flowers are potentially cross or self-pollinated, there is an extreme case of floral reduction in cleistogamous (CL) flowers (Richards 1997), which are obligatorily selfed. These occur in at least 256 species from 56 angiosperm families (Lord 1981). In 'true' cleistogamous species (Plitmann 1995) both open, potentially cross-pollinated flowers (chasmogamous flowers, CH) and CL flowers may develop on the same individual. The CH-CL system provides a mixed-mating system with both potentially variable (CH) and relatively invariable (CL) offspring. There are only few studies that explicitly differentiated between pollination mode and floral types (e.g., Culley 2000), while in most cases CH flowers were simply assumed to be outcrossed.

The study species are characteristic elements of species rich floodplain meadows of the alliances *Cnidion* and *Molinion*. The restoration of these meadows through transfer of

plant material from species-rich source stands is the aim of extensive current conservation projects (Donath *et al.* 2003; Hölzel & Otte 2003). Since this could lead to the establishment of new, initially small populations consisting of individuals from different source populations, the aim of **Chapter 5** was to investigate possible consequences of pollen source and floral type on seed production, seed traits and offspring fitness of the study species in two different experimental environments. We carried out three experiments:

- **Experiment I**: the effects of self-pollination *vs.* possible cross pollination by insects on the production of CH capsules and CH seed set was studied by comparing plants that were enclosed in perforated cellophane bags to exclude pollinators with an untreated control.
- **Experiment II**: to test for effects of pollen source on seed number and seed mass, individual flowers were hand-pollinated either with (i) pollen from the same flower (self-pollination), (ii) pollen from another plant from the same population (potentially cross-pollinated) or (iii) from another population (cross-pollinated). Additionally, (iv) seeds from CL capsules were included into the analysis.
- **Experiment III**: to study the effects of pollen source on offspring growth fitness under environmental conditions that resemble those of conspecifics (as concerns the microbial environment) and control conditions, we created two experimental environments. When Experiment I was finished we collected, for each species separately, the soil from the pots. Half of the soil was sterilised at 115° C for 24 h and the other half remained untreated. Then either 31.8 cm3 of sterilised or unsterilised soil (3.2% of the final soil volume) was added to new pots filled with commercial potting soil and mixed. Individual seedlings of each species, originating from seeds of different pollen source (Experiment II), were transplanted into these pots filled with sterilised or unsterilised soil of the same species.

The production of CH and CL capsules was temporarily separated in the study species, with CL capsules emerging after cessation of CH flowering. It is very likely that production of the different floral types is triggered by different environmental clues (Le Corff 1993) and that species with non-overlapping production of floral types may regulate the production of CL capsules according to the reproductive success of the CH flowers (Redbo-Torstensson & Berg 1995).

Pollinator exclusion had little effect on the production of CH capsules and seeds in the study species. Abortion of CH capsules was about 90% in *V. elatior* and *V. pumila*, while CL capsules produced abundant seeds. Therefore, seeds from CH flowers may contribute only a small fraction of total seed production, but may be more important in certain years. The fact that mass per seed of *V. pumila* estimated using capsules from

field populations (Hölzel & Otte 2004b) were identical with the values for CL seeds from the present study (1.08 mg in both cases) suggests that the majority of ripe seeds in natural populations may be from CL pollination.

In both species, seedling growth was significantly reduced under 'home'-conditions (**Chapter 5**). This effect is most probably owing to negative effects of the microbial soil community, which has been demonstrated for a large number of plant species (Bever 1994; Packer & Clay 2000; Klironomos 2002). Though we did not find general interactions between pollen source and soil treatment, our results still suggest that under stressful conditions (i.e., with a conspecific microbial community) relative fitness of selfed offspring of *V. stagnina* was significantly higher than that of crossed progeny. Recombination between distant populations apparently resulted in genotypes that showed signs of outbreeding depression under stressful conditions.

In accordance with our results (**Chapter 5**), the number of seeds per capsule did not differ between pollen sources in a large population of *Pedicularis palustris*, however, autogamous selfing in a small population resulted in reduced seed production (Karrenberg & Jensen 2000). In three European violet species there were no differences in seed abortion between CH and CL capsules (Berg & Redbo-Torstensson 1999), but it is not clear whether CH flowers were actually cross-pollinated in that study.

Judging from seedling biomass reached after eight weeks, there was no indication of inbreeding depression in the study species (**Chapter 5**). In *V. stagnina*, relative fitness varied between soil treatments, but on average fitness did not differ between pollination modes or floral types. Similarly, no indication of inbreeding depression in three other *Viola* species was found by Berg and Redbo-Torstensson (1999). This may suggest that deleterious alleles that may become homozygous as a consequence of selfing have been purged out of the populations during the phylogenetic history of the study species. Similarly, in *Viola canadensis* relative fitness of selfed and outcrossed offspring varied among plant traits but inbreeding depression and differences between floral types were generally low (Culley 2000).

The fact that *V. stagnina* plants pollinated with pollen from another population had a significantly lower relative fitness than plants pollinated by pollen of the same population under 'home'-conditions (**Chapter 5**) suggest that there may be outbreeding depression among distant populations. Indications for outbreeding depression were also

found for *Gentianella germanica*, where crosses within the same population performed better than inter-population crosses (Fischer & Matthies 1997).

Since populations of *V. stagnina* are highly isolated in the study area (**Chapters 3, 6**), gene flow is very low and consequently genetic differentiation among populations rather high (**Chapter 6**). Therefore, crossings between these populations may lead to biochemical or physiological incompatibilities between genes or the breaking of coadapted gene complexes (Dobzhansky 1972; Siikamäki 1999) of these populations and to lowered fitness especially under stressful conditions. Outbreeding depression is of great concern in conservation biology where efforts are undertaken to restore genetic diversity and heterozygosity of rare plant species (Dudash & Fenster 2000; Fenster & Galloway 2000; Montalvo & Ellstrand 2001; Edmands & Timmermann 2003). Our data suggest that the creation of initially small founder populations through the transfer of plant material in restoration projects, which may lead to increased probabilities of selfing, will not directly affect population viability of these rare species. However, in new populations consisting of genotypes from different source stands, outbreeding depression as the result of cross-pollination may impair seedling growth and population viability. Therefore, mixing the donor material from different populations should be avoided.

Conclusions (3)

- **The study species are probably not pollen limited since pollinator exclusion had only small effects on CH capsule production. In general, plants produced much more CL than CH capsules.**
- **Seed mass and numbers did not differ significantly among pollen sources. However, CL seeds were significantly smaller than CH seeds.**
- **Seedling growth was reduced in plants grown under 'home'-conditions, i.e. with the microbial community of the same species, as compared to control soils.**
- **Under 'home'-conditions, relative fitness of selfed seedlings of *V. stagnina* was significantly higher than that of crossed progeny. High genetic differentiation among populations as a consequence of isolation may result in outbreeding depression in this species.**
- **There were no indications for inbreeding depression in the study species.**
- **The creation of initially small founder populations through the transfer of plant material in restoration projects will not directly affect population viability of these rare species. However, mixing the donor material from different populations should be avoided to prevent possible outbreeding depression.**

D. Identification of sensitive stages in the life-cycle for conservation

‹*Chapter 4*›

The growth and viability of populations ultimately depend on the vital rates: *survival*, *growth* and *fecundity* (i.e. *reproduction*; cf. Caswell 2001). Environmental conditions (both abiotic conditions and biotic interactions) exert selective forces that may lead to the development of specific suites of traits (e.g., Chapin *et al.* 1993) in certain habitat types, along successional trajectories or along clines from the centre to the margin of the species range (Sagarin & Gaines 2002). Since the future fate of organisms varies with age or stage, the demographic approach to conservation, i.e. population biological analyses of various aspects of the life-cycle as the fundamental unit for the description of organisms (Caswell 2001), may supply the necessary information for the conservation of rare and endangered species (e.g., Crouse *et al.* 1987; Caswell & Brault 1992; Schemske *et al.* 1994; Menges 2000; Pfab & Witkowski 2000; Caswell 2001; Morris & Doak 2002; Nicolè *et al.* 2005). The identification of sensitive stages in the life-cycle of endangered species is central in conservation biology (Caswell 2000, 2001; Morris & Doak 2002). One classical example is the study of Crouse *et al.* (1987) on the life-cycle of the threatened Loggerhead sea turtle (*Caretta caretta*), which based on population models and elasticity analysis redirected conservation effort away from eggs towards large juveniles and adults. The implementation of turtle excluder devices on shrimp-trawlers in 1987 that allowed turtles to escape from the fishing nets was influenced by this demographic study (Caswell & Brault 1992).

There are two kinds of analyses for identification of the 'importance' of life-cycle stages to population growth (Horvitz *et al.* 1997; Caswell 2000, 2001), (i) *prospective* analysis, i.e. sensitivity and elasticity, and (ii) *retrospective* analysis, i.e. life table response experiments (LTRE).

Prospective analyses look forward and explore the effects of variation in vital rates on population growth rate (λ). They are independent of actual observed variation in vital rates but can, for example, also quantify the effects of impossible changes in the life-cycle (e.g., '*what would happen, if pigs had wings?*', Horvitz *et al.* 1997; Caswell 2000). Prospective analyses neither depend on information on how vital rates varied in the past, presently or in the future, nor does knowledge of this variation contribute to this kind of analysis. *Sensitivity* ($\mathbf{s}_{ij}$) represents the slope of λ as a function of absolute

changes in single matrix entry (a_{ij}), keeping all other entries constant (for the equation of sensitivity cf. Caswell 2001). Since matrices contain transition probabilities that range between 0 and 1 and fecundities that are measured on a different scale, one can also consider the response of λ to *proportional* rather than *absolute* changes in matrix entries. This is done by analysis of *elasticity* ($\mathbf{e}_{ij}$), which represents the slope of *log* λ as a function of *log* a_{ij} (de Kroon *et al.* 1986; Caswell 2001). Elasticity is related to sensitivity by the equation $\mathbf{e}_{ij} = \mathbf{s}_{ij} \times (a_{ij} / \lambda)$. Across a matrix elasticities sum up to unity.

Retrospective analyses look backward, addressing the question of how observed variation in transitions has contributed to observed variation in λ (Caswell 2000). Hence they are concerned with *variation* in λ as a function of *variation* in vital rates, and as such knowledge of this variation is essential. LTRE has some analogies with analysis of variance (ANOVA) because it is based on linear models of λ as a function of 'treatments', relies on the partitioning of effects, and allows the analysis of multi-factorial (fixed or random effect) designs. However, LTRE unlike ANOVA is not concerned with the presence of statistically significant differences in λ or vital rates. Rather, even infinitesimal small differences in λ can be analysed for the vital rates underlying this difference (for an example see Caswell 1996). In LTRE, contributions of life-cycle transitions ($\mathbf{c}_{ij}$) to variation in λ are expressed as the product of the difference of the respective entries in two matrices compared (Δa_{ij}) and the sensitivity of λ towards this element ($\mathbf{s}_{ij}$), evaluated half-way between the compared matrices (Caswell 1996, 2001). Consequently, matrix transitions that do not vary and those with a low impact on λ will also make no or only a small contribution to differences in λ.

In conservation studies based on an average matrix for a species, variance or standard deviation of respective a_{ij} are used instead of Δa_{ij}. (e.g., Ehrlén & van Groenendael 1998). However, Caswell (2000, 2001) refuted that retrospective analysis could be used to identify sensitive stages in the life-cycle, because a matrix entry may have a small contribution to variation in λ because (i) a_{ij} is physiologically or developmentally constrained (Caswell 2001), or (ii) the variation of a_{ij} is small for other, e.g. environmental, reasons. In the former case a_{ij} would be a bad target for management, whereas in the latter case management could positively influence variation in a_{ij}. Only prospective analysis that explores functional relationships between transitions and population growth can identify those vital rates that have a large influence on λ. Whether or not these vital rates or transitions can be changed through management will

depend on the life-cycle and biology of the species in question. However, retrospective analysis can be used in conservation biology to identify causes for the decline of rare species. Caswell (2001) identified four tasks in demographic conservation biology that can be sorted out by use of the appropriate demographic methods (Table 7.1).

Table 7.1 Tasks and methods of demographic analyses within conservation biology (Caswell 2001).

Task	**Method**
Assessing population status	Estimation of λ and its uncertainty
Diagnosing causes of decline	Retrospective perturbation analysis (LTRE)
Prescribing management tactics	Prospective perturbation analysis (elasticity, sensitivity)
Prognosis of population fate	Population projection

The study species are among the most endangered species across Europe deserving special conservation efforts and red-listed in many European countries (e.g., Korneck *et al.* 1996; Schnittler & Günther 1999). The species, which are today restricted mainly to the valleys of large lowland rivers (Burkart 2001), have probably been more widespread after the last glaciation, but retreated to floodplains, which provide regionally sub-continental conditions, flood disturbances, and mostly non-intensive human land use. An analysis of grid maps suggests that the species have undergone a severe decline by about 50% during the last decades (Eckstein *et al.* 2006a), which caused strong fragmentation and isolation of populations (**Chapter 3, 6**). As such they belong to the 'new rares' (Oostermeijer 1996), which appear to be more vulnerable to isolation and range contractions than 'naturally' rare plants (Huenneke 1991). Two aims of the study presented in **Chapter 4** were thus to try to identify sensitive stages in the life-cycles and to identify and evaluate possible threats to population viability and species conservation.

The analysis was based on the fate of marked plants in permanent plots of 27 populations from Dyje and Rhine floodplains. Information of three to six populations per species-region combination was pooled to obtain robust 5×5 Levkovitch transition matrices, based on five life-cycle stages. Using matrix calculations (cf. Caswell 2001) we obtained the elasticities of population growth rate to single matrix transitions, vital rates and life-cycle stages. To analyse differences in population growth between species and regions we applied a two-way fixed factor LTRE.

Elasticity analysis revealed that despite some differences between regions, small vegetative and small and large generative plants contributed most to λ in *V. elatior*, whereas all four adult stages contributed roughly similar to λ in *V. pumila* (**Chapter 4**). These two species also showed similar elasticities with respect to their vital rates (Table 7.2). In contrast, the contribution of different stages and vital rates varied strongly between regions in *V. stagnina*. Dyje populations of this species had the highest elasticity for survival, whereas in Rhine populations showed the highest elasticity for fecundity of all species-region combinations and the lowest elasticity for survival. Generally, the elasticities of the study species were in a range typical of polycarpic perennials of open habitats (Silvertown *et al.* 1993). Large variation in elasticity between regions in *V. stagnina* was probably related to the variation in population growth rate.

Table 7.2. Elasticity (**e**) of population growth rate of *Viola elatior*, *V. pumila* and *V. stagnina* from two regions (Dyje, Rhine) to survival, growth and fecundity.

Species	Region	**e** (survival)	**e** (growth)	**e** (fecundity)
V. elatior	Dyje	0.4546	0.4128	0.1326
	Rhine	0.4672	0.4054	0.1274
V. pumila	Dyje	0.5366	0.3669	0.0965
	Rhine	0.4648	0.4312	0.1040
V. stagnina	Dyje	0.6527	0.3355	0.0119
	Rhine	0.3157	0.4845	0.1998

In growing populations the contributions of growth and fecundity tend to increase, which has been shown for *Gentiana pneumonanthe* (Oostermeijer *et al.* 1996), and for *Cirsium vulgare* and *Pedicularis furbishiae* (Silvertown *et al.* 1996). Elasticities of vital rates varied between years and populations in *Agrimonia eupatoria* and *Geum rivale* (Kiviniemi 2002), *Primula vulgaris* (Valverde & Silvertown 1998), and *Heracleum mantegazzianum* (Hüls 2005; Hüls *et al.* 2007), whereas they were relatively constant in three species of *Pinguicula* (Svensson *et al.* 1993). The fact that matrix transitions are not independent of each other and that elasticity varies with population growth rate (Oostermeijer *et al.* 1996; Silvertown *et al.* 1996; Caswell 2001) makes it difficult to identify unique stages or vital rates as sensitive phases or processes for conservation or management. At least the results of elasticity analyses have to be interpreted with caution. Additionally, since elasticity is calculated as sensitivity multiplied by the quotient of the transition value (a_{ij})

and λ (de Kroon *et al.* 1986; Caswell 2001) common transitions will often also have a high elasticity, whereas rare transitions may be better targets for conservation or recovery plans.

For example, fecundity in *V. stagnina* from the Dyje floodplains was already very low owing to low habitat quality (missing or infrequent management, cf. **Chapter 3, 4**), which, in turn, led to elasticities close to zero, indicating that this transition (and the seedling stage) did not contribute significantly to λ. However, these populations will probably not resume positive growth unless reproduction and germination are enhanced through changes in management or disturbances improving conditions for the germination of seeds from the seed rain or the persistent seed bank.

A persistent soil seed bank, i.e. a reservoir of dormant seeds that remain viable over a number of germination seasons, is a common feature of many plant species over a wide range of plant life histories, habitats and geographic regions (Leck *et al.* 1989; Baskin & Baskin 2001). Theoretical models suggest a number of possible effects of a soil seed bank on plant demography, population genetic structure and evolutionary biology. Given the large temporal (year-to-year) and spatial variation of climatic conditions and of the intensity of biotic interactions, a persistent soil seed bank may present a means to avoid or escape unfavourable conditions, and to buffer population growth rate against environmental stochasticity especially in dynamic or cyclic-successional habitats. If seed banks can disperse genes through time they may conserve genotypes in a population over periods in which they are selected against. Consequently, the rate of evolution may be damped if the existence of persistent dormant seeds creates a lag between the year in which selection takes place and the time the response to selection is visible (for copepods: Hairston & De Stasio 1988). Furthermore, persistent seed banks may counteract the consequences of random genetic drift by pooling seeds of many generations and averaging out each generation's allele frequency sampling error, especially in small populations. Alternatively, differential selection between dormant seeds in the soil and the extant green plants above-ground may also lead to variation in genetic structure of different life-cycle stages. If seeds accumulate mutations as they lie dormant in the soil, the seed bank could also function as a source of genetic novelty (Levin 1990). The possible role of persistent seed banks as a means of escape in time and space has been shown for annual plants in the models of Cohen (1966) Although a few empirical studies since the early 1990s have dealt with the demographic consequences of

a seed bank for annual and short-lived plant species (Kalisz 1991; Kalisz & McPeek 1993), our current understanding of the potential role of soil seed banks for ecological and evolutionary dynamics of aboveground populations largely comes from theoretical studies and mathematical models (e.g., Templeton & Levin 1979; Venable & Lawlor 1980). There is evidence that supports some of the proposed theoretical ideas. For example, some empirical studies suggest that the seed bank may serve as a reservoir of genetic variation at a very local level (Tonsor *et al.* 1993) and slows population differentiation (McCue & Holtsford 1998). A decrease in homozygosity from the seed bank to adults indicated inbreeding depression acting on post-emergence traits or overdominance effects, i.e. heterozygote advantage (Tonsor *et al.* 1993). The studies of McGraw and co-workers have shown genetic divergence between populations originating from young and old soil seeds and extant surface plants (Bennington *et al.* 1991; McGraw *et al.* 1991; McGraw 1993; Vavrek *et al.* 1991). Plants derived from readily germinating seeds differed in survival and growth from plants derived from late germinating seeds in the short-lived *Lesquerella fendleri* (Cabin *et al.* 1996).

The presence of a persistent seed bank in the study species (Hölzel & Otte 2004a; Eckstein *et al.* 2006a) may probably buffer populations from environmental stochasticity and extinction (e.g., Solbrig *et al.* 1988; McCue & Holtsford 1998; Cabin & Marshall 2000; Adams *et al.* 2005). Consequently, the floodplain violets may show strong extension and contraction of population size along successional trajectories, starting with a burst of germination after a major disturbance. With the density of adults increasing, germination and seedling survival decrease and population size slowly decreases with time until populations only persist as seeds in the soil seed bank. This has been described as remnant regional population dynamics (Eriksson 1996).

In comparison with the overall mean matrix (λ=0.9085), populations across regions of *V. elatior* (λ=0.8602) and *V. pumila* (λ=0.8261) had lower growth rates. These differences were accounted for by negative contributions of growth and fecundity (**Chapter 4**). Higher λ of *V. stagnina* across regions (λ=1.0565) was owing to positive contributions of growth and fecundity.

Across species, the Dyje and the Rhine populations had population growth rates of 0.8464 and 0.9294, respectively. Survival did not contribute to the difference in population growth between the overall mean matrix and the regional matrices across species. Negative contributions of growth transitions were outweighed by positive

contributions of fecundity in the Rhine populations, whereas the pattern was exactly opposite in the Dyje populations (**Chapter 4**). In summary the high performance of the Rhine populations of *V. stagnina* had a strong influence on both the mean λ of this species across regions and on the mean λ of Rhine populations across species. Especially fecundity contributed to the difference in λ between the Dyje and Rhine populations of *V. stagnina*.

Conclusions (4)

- **Differences in population growth rate (λ) between species across regions were accounted for by higher growth and fecundity of *V. stagnina* in comparison with *V. elatior* and *V. pumila*.**
- **Differences between regions were owing to lower growth and higher fecundity of Rhine populations.**
- **These patterns were driven by large differences between Rhine and Dyje populations of *V. stagnina*.**
- **Also elasticity of λ to different vital rates or life-cycle stages varied strongly in *V. stagnina*, whereas it was relatively similar in *V. elatior* and *V. pumila*.**
- **In the latter two species, growth and survival were most important for λ. Related to large variation in λ between regions, in *V. stagnina* survival was most important in Dyje populations and elasticities of growth and fecundity were high in Rhine populations. This makes it difficult to identify unique targets for conservation in the latter species.**
- **Low habitat quality in populations along the Thaya causes an almost complete failure of germination in many populations. The resulting very low probabilities for these transitions preclude the identification of important life-cycle stage for conservation by simple elasticity analysis.**
- **For the conservation of the three species along the Rhine the continuation of the present management aiming at increasing the survival of vegetative plants will be necessary. Conservation measures along the Thaya should primarily aim to improve habitat quality by introduction of regular management and include an intensive monitoring of the extant populations to control the success of the conservation strategy.**
- **The presence of a persistent seed bank in the study species may be a buffer against environmental stochasticity and extinction, and lead to remnant regional population dynamics in these floodplain violets.**

Chapter 8

Ausführliche Zusammenfassung und Schlussfolgerungen.

Im Folgenden werden die wichtigsten Ergebnisse der einzelnen Studien, aus denen die vorgelegte Arbeit besteht, ausführlich dargestellt und vor dem Hintergrund der in der Einleitung (Kapitel 1, S. 6) formulierten Ziele und Fragestellungen zusammenfassend diskutiert. Für Detailergebnisse einzelner Publikationen, Abbildungen und Tabellen wird auf die jeweiligen Kapitel (**Chapter 2-6**) verwiesen.

Die vorliegende Arbeit beschäftigt sich mit drei europaweit gefährdeten Arten der Gattung *Viola*, *V. elatior* Fries, *V. pumila* Chaix und *V. stagnina* Kit. (Korneck *et al.* 1996; Schnittler & Günther 1999; Eckstein *et al.* 2006a). Die drei Arten zeigen ein kontinentales Verbreitungsmuster (Hultén & Fries 1986) mit einem Verbreitungszentrum in der gemäßigten Klimazone Osteuropas und Westsibiriens und erreichen in Zentral- und Westeuropa die Westgrenze ihres Verbreitungsgebiets. Es handelt sich um iteropare Hemikryptophyten mit einem komplexen Lebenszyklus, einem gemischten Bestäubungssystem aus chasmogamen und kleistogamen Blüten und einer ausdauernden Samenbank.
Die vorliegende Arbeit verwendet einen experimentellen demographischen Ansatz zur Untersuchung verschiedener Aspekte der Populationsbiologie und des Artenschutzes der drei Stromtalveilchen. Die spezifischen Teilziele der Arbeit waren

A. den Einfluss von (vgl. Fig. 1.1)
   - Umweltstochastizität, demographischer und genetischer Stochastizität und
   - deterministischen Prozessen (Landnutzung, Habiatfragmentierung) auf das Populationswachstum und die Überlebens- und Entwicklungsfähigkeit der Populationen zu untersuchen,

B. den Lebenszyklus der Arten in zentralen und peripheren Populationen zu beschreiben und zu vergleichen, die genetische Populationsstruktur zu erfassen

und die relative Bedeutung von genetischer Drift und Genfluss in zentralen und peripheren Populationen abzuschätzen,

C. die Bedeutung des Bestäubungssystems und der Pollenquelle für die Samenproduktion der Mutterpflanzen und die Fitness der Nachkommen zu analysieren und

D. sensible Stadien im Lebenszyklus der Arten zu identifizieren, um Informationen über geeignete Pflegemaßnahmen abzuleiten.

A. Einfluss stochastischer und deterministischer Prozesse auf die Überlebensfähigkeit der Populationen

‹Kapitel 2, 3, 4, 6›

In **Kapitel 2** wurden in Keimschalen unter identischen Bedingungen vorgezogene Keimlinge von *V. elatior*, *V. pumila* und *V. stagnina* auf einem Versuchsgelände der Universität Gießen einzeln in die Mitte von jeweils 0.1 m² großen Versuchsflächen gepflanzt. Die Versuchsflächen wurden im Experiment einer der folgenden vier Kombinationen der Faktoren „*zwischenartliche Konkurrenz*“ (C von engl. ***clipping***)) und „*Wasserverfügbarkeit*“ (W) zugeordnet:

- C+W+, umgebende Vegetation entfernt, Bewässerung,
- C+W-, umgebende Vegetation entfernt, keine zusätzliche Bewässerung,
- C-W+, umgebende Vegetation nicht entfernt, Bewässerung, und
- C-W-, umgebende Vegetation nicht entfernt, keine zusätzliche Bewässerung.

Bei den C+ Behandlungen wurden die oberirdische diffuse Konkurrenz durch Arten der umgebenden Grünlandvegetation (vornehmlich Gräser) durch wiederholtes Abschneiden über den gesamten Versuchszeitraum von 127 Tagen beseitigt, während die Wasserversorgung entweder durch Zugabe von 1 Liter Wasser alle zwei Tage verbessert wurde oder der natürlichen Versorgung durch Niederschläge entsprach. Bei den C- Behandlungen erfuhren die Keimlinge oberirdische Konkurrenz durch die sie umgebende Grünlandvegetation und wurden entweder extra bewässert oder nicht. Die Versuchsflächen befanden sich in drei Grünlandtypen, die aufgrund ihrer Position an einem leicht geneigten Hang und die vorhandene Bodenart einen Gradienten der Wasserverfügbarkeit repräsentierten. Das Experiment bestand somit aus der faktoriellen Kombination von drei Grünlandtypen (trocken, mittel, feucht), drei Arten (*V. elatior*,

V. pumila und *V stagnina*), zwei Konkurrenzvarianten und zwei Bewässerungsvarianten mit jeweils fünf Wiederholungen pro Kombination.

Das Schicksal jedes Keimlings (lebendig oder abgestorben) wurde zu zehn Zeitpunkten zwischen dem 19. Mai und dem 23. September erfasst. Zusätzlich wurden bei diesen Gelegenheiten Höhe, Anzahl der Blätter sowie Anzahl der Samenkapseln (kleistogame Reproduktion) jeder Pflanze erhoben. Diese Daten dienten zur separaten Analyse der Effekte von Konkurrenz und der Variation von Umweltbedingungen (d. h. *Umweltstochastizität*) auf (i) Überleben, (ii) Wachstum und (iii) Reproduktion der Pflanzen.

Die Ergebnisse zeigten, dass Überleben, Wachstum und Reproduktion unterschiedlich auf zwischenartliche Konkurrenz und Wasserverfügbarkeit reagierten. Eine Überlebensanalyse (siehe Fox 1993) machte deutlich, dass (1) die kumulative Überlebensrate der Keimlinge im Feuchtgrünland signifikant höher war als in den beiden anderen Grünlandtypen, und dass (2) die Überlebensrate der Keimlinge auf Versuchsflächen mit zusätzlicher Bewässerung signifikant höher war als in nicht bewässerten Flächen. Folglich hatte Wasserversorgung den größten Einfluss auf die Überlebenswahrscheinlichkeit der Keimlinge. Die entscheidende Rolle der Wasserversorgung für Samenkeimung und Keimlingsüberleben wurde z. B. auch in den Untersuchungen von De Jong und Klinkhamer (1988) sowie Xiong *et al.* (2003) gezeigt. Bei zwei biennen Pflanzenarten aus Dünensystemen war die Sterberate der Keimlinge signifikant mit dem Bodenwassergehalt korreliert und nahm, wie in der vorliegenden Arbeit (**Kapitel 2**), deutlich zu, wenn der Wassergehalt unter 5% des Bodentrockengewichts sank (De Jong & Klinkhamer 1988).

Grünlandtyp, Bewässerung und Arteffekte erklärten den größten Teil der Varianz in der Größenzuwachsrate (HGR), während die Blattakkumulationsrate (LGR) und die Reproduktion in der Rangfolge abnehmender Bedeutung durch die Faktoren „*Grünlandtyp*“, „*Konkurrenz*“ und „*Bewässerung*“ beeinflusst wurden. Bewässerung allein war nicht hinreichend für die Produktion von Samenkapseln im trockenen und mittleren Grünland, denn bewässerte Pflanzen bildeten hier nur dann kleistogame Samenkapseln aus, wenn gleichzeitig die umgebende Vegetation entfernt worden war. Im feuchten Grünland war die Kapselproduktion größer auf gemähten als auf ungemähten Versuchsflächen.

In **Kapitel 3** wurde die Populationsstadienstruktur der drei behandelten Arten auf insgesamt 122 Versuchsflächen von 0.1 m^2 Flächengröße in 27 natürlichen Populationen aus zwei Regionen (Hessischer Oberrhein, Deutschland; March-Thaya Auen, Tschechien) untersucht. Alle Individuen wurden im Jahr 2001 markiert und aufgrund des Vorhandenseins von Blüten und der Anzahl der Sprosse (Rameten) einem der fünf folgenden Entwicklungsstadien zugeteilt: (1) Keimlinge, (2) kleine vegetative, (3) große vegetative Pflanzen, (4) kleine blühende und (5) große blühenden Pflanzen (vgl. **Kapitel 3**, *Material and Methods*, *Life-cycle stages*). Im Jahre 2002 wurden die Versuchsflächen wieder aufgesucht und alle Pflanzen erneut aufgenommen. Unter Verwendung varianzanalytischer Methoden (ANOVA; Quinn & Keough 2002) wurden Unterschiede zwischen „*Arten*", „*Regionen*" (für die Diskussion von Unterschieden zwischen zentralen und peripheren Populationen vgl. unten) und „*Untersuchungsjahren*" (d. h. *Umweltstochastizität*) auf die ***Dichte der Entwicklungsstadien*** analysiert. Zusätzlich wurden mit Hilfe zweier log-linearer Modelle (Quinn & Keough 2002) die Effekte von „*Untersuchungsjahr*" (zeitliche Variation) und „*Nutzung*" (regelmäßig, unregelmäßig) sowie „*Untersuchungsjahr*" (zeitliche Variation) und „*Population*" (räumliche Variation) auf die ***Stadienhäufigkeit*** untersucht.

Die Keimlingsdichte unterschied sich signifikant zwischen den beiden Regionen und den Untersuchungsjahren (**Kapitel 3**), wohingegen sich die Dichte kleiner und großer vegetativer Pflanzen nur signifikant zwischen den Arten unterschied. Bei allen drei Arten variierte die Häufigkeit der Entwicklungsstadien, d. h. die Stadienstruktur der Populationen (Oostermeijer *et al.* 1994a), signifikant zwischen Jahren (*Umwelt-stochastizität*) und den Regionen. Signifikante Unterschiede zwischen den untersuchten Regionen spiegeln vermutlich vor allem Unterschiede in der Habitatqualität wider. Ein direkter Vergleich des Einflusses der Faktoren „*Untersuchungsjahr*" und „*Nutzung*" (regelmäßig oder unregelmäßig) zeigte, dass bei *V. elatior* und *V. stagnina* beide Faktoren ähnliche große, signifikante Effekte auf die Stadienstruktur der Populationen hatten, während bei *V. pumila* die Häufigkeit der Stadien nur signifikant zwischen den Untersuchungsjahren schwankte (**Kapitel 3**).

Durch die Tatsache, dass sich alle Populationen am Hessischen Oberrhein ausschließlich in Naturschutzgebieten befanden oder auf Flächen, die im Rahmen von Agrarumweltprogrammen bewirtschaftet werden, während die Populationen in den March-Thaya Auen überwiegend unregelmäßig genutzt werden, war es schwierig die

Effekte von Nutzung und Region eindeutig zu trennen. Allerdings variierten, nachdem die Populationen entweder nach der Region (Rhein *vs*. March-Thaya Auen) oder nach der Nutzung (regelmäßig *vs*. unregelmäßig) gruppiert wurden, bei *V. elatior* und *V. stagnina* die Anzahl der Keimlinge deutlich stärker zwischen genutzten und unregelmäßig genutzten Populationen (über die beiden Regionen) als zwischen den Regionen (über die Nutzungsvarianten). Die deutet an, dass die Nutzung vermutlich einen größeren Effekt auf die Populationsstruktur hat als die geographische Lage mit allen Faktoren, die sich zwischen den Regionen unterscheiden. Bei *V. pumila* waren die Unterschiede zwischen Regionen oder Nutzungen eher gering.

Diese Befunde werden durch die in **Kapitel 4** präsentierten Untersuchungen zur Populationsdynamik der drei Arten bestätigt. Hier wurden mit Hilfe von Daten zum Schicksal markierter Individuen von Dauerbeobachtungsflächen aus 27 Populationen Lebenszyklen der untersuchten Arten entwickelt. Um robuste 5×5 Levkovitch Übergangsmatrizen auf der Basis der genannten fünf Entwicklungsstadien zu erhalten, wurden die Informationen von drei bis sechs Populationen pro Art-Region Kombination vereinigt. Mit Hilfe von Matrixmodellierung (vgl. Caswell 2001) wurden die beobachtete intrinsische Wachstumsrate der Populationen (λ_O) sowie mit Hilfe der Bootstrap-Technik (vgl. Dixon 1993) eine Bootstrap-Wachstumsrate (λ_B), die beobachtete und die stabile Stadienverteilung unter Gleichgewichtsbedingungen, die Netto-Reproduktionsrate (R0), die Generationszeit und das Durchschnittsalter der Pflanzen bei der ersten Reproduktion ermittelt.

Die beobachtete Populationswachstumsrate aller Art-Region Kombinationen mit Ausnahme von *V. stagnina* am Hessischen Oberrhein waren kleiner als eins. Allerdings überlappten die zu λ_B ermittelten 95%-Konfidenzintervalle, so dass sich λ_B nicht signifikant zwischen Arten oder Regionen unterschied. Da λ stark von der Witterung beeinflusst ist, deutet dieses Ergebnis an, dass sich die klimatischen Bedingungen im Untersuchungszeitraum nicht wesentlich (zumindest nicht im Hinblick auf ihre Auswirkungen auf λ) zwischen den Regionen unterschieden. Langfristig kann, solange die Populationen nicht aussterben, bei ausdauernden Arten ein λ von etwa eins erwartet werden (z. B. Solbrig *et al.* 1980; Newell *et al.* 1981; Silvertown *et al.* 1993; Svensson *et al.* 1993; Oostermeijer *et al.* 1996; Nicolè *et al.* 2005).

Trotz einer höheren durchschnittlichen Überlebens- und Reproduktionswahrscheinlichkeit von Pflanzen der March-Thaya Auen, war R0 von Populationen am

Hessischen Oberrhein so groß wie oder größer als in Tschechischen Populationen. Dies könnte ein Ergebnis von Unterschieden in der Habitatqualität zwischen den Regionen sein, die vor allem mit der Nutzung und dem Einfluss von wirbellosen Herbivoren zusammenhängen. In Populationen der March-Thaya Auen war ein großer jedoch stark variabler Anteil der Samenkapseln von den Larven von *Orbitis cyaneus* (L.), einem Käfer aus der Familie der Rüsselkäfer (Curculionidae) befallen, während dieses Phänomen in Populationen am Hessischen Oberrhein während des Untersuchungszeitraum nicht beobachtet wurde. Mit Hilfe markierter Pflanzen gewonnene Daten zum Befall deuten darauf hin, dass die Insekten lokal die Samenproduktion wesentlich reduzieren können. Zusätzlich führt das Fehlen einer regelmäßigen Nutzung der Stromtalwiesen in den March-Thaya Auen zur Ansammlung von Pflanzenstreu und der Entwicklung dichter Moosmatten. Im Gegensatz zur förderlichen Wirkung geringer Mengen von Streu über ausgestreuten Samen (Eckstein & Donath 2005) sind der Keimungsprozentsatz und der Etablierungserfolg von Keimlingen aus Samen auf einer dichten Streuschicht signifikant herabgesetzt (Facelli & Pickett 1991 und weitere Zitate in dieser Arbeit).

Die Analyse der genetischen Populationsstruktur und der Bedeutung von genetischer Drift, d. h. *genetischer Stochastizität*, in insgesamt 50 Populationen der untersuchten Arten (**Kapitel 6**) mit Hilfe von AFLP Markern (***a**mplified **f**ragment **l**ength **p**olymorphism*, vgl. Mueller & Wolfenbarger 1999) ergab, dass genetische Drift in Populationen von *V. pumila* and *V. stagnina* am Hessischen Oberrhein eine *relativ größere* Bedeutung hatte als Genfluss. Allerdings waren diese beiden Prozesse in keiner Art-Region Kombination im Gleichgewicht und genetische Diversität war nicht mit der Populationsgröße korreliert. Unterschiede zwischen den Regionen erklärten bei jeder der drei Arten einen relativ kleinen aber signifikanten Teil (etwa 6%) der gesamten genetischen Varianz des Datensatzes. Eine konsistent höhere Populationswachstumsrate in Populationen am Hessischen Oberrhein trotz geringerer genetischer Diversität deutet an, dass genetische Stochastizität (genetische Drift) nicht unbedingt zu einer biologisch bedeutsamen Verminderung der Überlebensfähigkeit von genetisch weniger variablen Populationen führt. Auch hier spielt vermutlich die bessere Habitatqualität von Populationen am Oberrhein durch regelmäßige Nutzung eine entscheidende Rolle.

Deterministische Prozesse wie z. B. Landnutzung, Habitatverlust, Habitatfragmentierung und Sukzession wirken auf dem Landschaftsmaßstab (**Kapitel 1**, vgl. Fig. 1.1) und betreffen meistens die gesamte Population. Im Gegensatz dazu beeinflussen

stochastische Prozesse meist nur Individuen innerhalb lokaler Populationen. Unvorhersagbare Schwankungen der Umweltbedingungen führen zu Schwankungen der durchschnittlichen Überlebens-, Wachstums- und Reproduktionsrate von Jahr zu Jahr über alle Individuen in der Population. Umweltstochastizität und ihre Extreme, d. h. extrem schlechte (*Katastrophen*) oder extrem gute Jahre (*Gunstjahre*) führen oft zu engen Korrelationen zwischen den fundamentalen Lebensprozessen, denn Jahre mit günstigen Bedingungen für das Überleben sind gewöhnlich auch gute Jahre für Wachstum und Reproduktion und umgekehrt (Caswell 2000; Morris & Doak 2002). Im Gegensatz dazu beeinflussen demographische und genetische Stochastizität nur einzelne Individuen innerhalb der Population unabhängig von ihrem Entwicklungsstadium. Mit anderen Worten beeinflusst Umweltstochastizität (z. B. Witterung) große und kleine Populationen gleichermaßen, während die Auswirkungen demographischer und genetischer Stochastizität stark von der Populationsgröße abhängig sind. Da Zufallsschwankungen der tatsächlichen Realisation der durch eine Wahrscheinlichkeit quantifizierbaren individuellen Überlebens- oder Reproduktionsrate in großen Populationen ausgeglichen werden, d. h. einige Individuen verwirklichen eine höhere andere eine geringere als die durchschnittliche Überlebens- oder Reproduktionsrate, haben in kleinen Populationen demographische und genetische Stochastizität größere Auswirkungen als in individuenreichen Populationen.

Intensivierung der Landnutzung und Nutzungsaufgabe sind beide mit einer zunehmenden Fragmentierung von Habitaten und einer Verminderung der Habitatqualität verknüpft. Habitatfragmentierung führt durch Verminderung der Populationsgröße, vermehrte Randeffekte und Reduzierung der Kolonisationsrate zu einem erhöhten Aussterberisiko lokaler Populationen (Hanski 1999; Debinski & Holt 2000; Saunders *et al.* 2001; Eriksson *et al.* 2002). Unter der Annahme von zeitlicher Nischenkonstanz, waren die untersuchten Arten am Ende der letzten Eiszeit (Jüngere Dryasperiode, 12000 Jahre vor heute; vgl. Firbas 1949; Frenzel 1968), als die klimatischen Bedingungen in Europa deutlich stärker kontinental getönt waren, vermutlich weiter verbreitet und häufiger als derzeit. Mit der Entwicklung des heutigen Klimas und des zunehmenden anthropogenen Einflusses in der Landschaft zogen sie sich in Auenhabitate zurück, die sich durch (1) ein kontinental getöntes Regionalklima, (2) verminderte Konkurrenz wuchskräftiger Arten aufgrund regelmäßig wiederkehrender Überflutungsereignisse und (3) relativ geringen menschlichen Einfluss auszeichnen.

Eine Analyse der historischen Verbreitung in Mitteleuropa erbrachte Hinweise auf einen starken Rückgang aller drei Arten innerhalb der letzten Jahrzehnte (Eckstein *et al.* 2006a). Als „Neue Seltene" Arten (engl. '*new rares*' *sensu* Oostermeijer 1996) sind die untersuchten Arten demnach rezent verstärkter Habitatfragmentierung und Isolation lokaler Populationen ausgesetzt gewesen.

In Folge von Habitatfragmentierung reduziert sich die die Populationsdichte und die individuelle Fitness sowohl von häufigen als auch seltenen Arten (Widén 1993; Kéry *et al.* 2000; Hooftman *et al.* 2003; Lienert *et al.* 2002; Lienert & Fischer 2003; Vergeer *et al.* 2003a, b). Dichte, Stadienstruktur, Samenansatz und der Schädigungsgrad durch Herbivore bei *Primula farinosa*, einem in der Schweiz noch relativ häufigen Spezialisten von Nasswiesen und Niedermooren, wurden durch einen der drei Aspekte der Habitatfragmentierung, d. h. verringerte Populationsgröße, zunehmende Isolation und Randeffekte, beeinflusst (Lienert & Fischer 2003). Dabei ist die Reaktion auf Habitatfragmentierung artspezifisch (Fischer & Stöcklin 1997; Hooftman *et al.* 2003; Honnay *et al.* 2004; Lindborg *et al.* 2005), d. h. klonale Arten, Arten mit wenigen und/oder großen Diasporen oder Arten mit Diasporen ohne spezialisierte Ausbreitungsstrukturen sind stärker durch Isolation beeinträchtigt als Arten mit einem besseren Ausbreitungsvermögen (Kolb & Diekmann 2005).

In der vom Menschen geschaffenen und erhaltenen Kulturlandschaft ist ausgeprägte Habitatfragmentierung jedoch der Endpunkt und die Konsequenz einer Entwicklung, die mit anderen deterministischen Prozessen verknüpft ist, welche ihrerseits zur Extinktion lokaler Populationen beitragen und das Überleben von Populationen bedrohen können. Die meisten dieser Prozesse führen zu einer Verringerung der Populationsgröße durch die schleichende Verschlechterung der Habitatqualität (z. B. Eisto *et al.* 2000; Endels *et al.* 2002). Kleinere Populationen sind wiederum stärker von Extinktion bedroht als große (z. B. Fischer & Stöcklin 1997; Matthies *et al.* 2004).

Eine detaillierte Analyse der Roten Liste der gefährdeten Pflanzenarten in Deutschland zeigte, dass 66% von 819 ausgestorbenen oder gefährdeten Taxa durch Habitatzerstörung, zumeist Bautätigkeiten (Straßen und andere Infrastruktur) sowie das Wachstum von Dörfern, Städten und Industrieanlagen bedroht sind (Korneck *et al.* 1998). Der zweitwichtigste Faktor, der 56% der Arten betrifft, ist die landwirtschaftliche Nutzung. Interessanterweise wirken sich landwirtschaftliche Intensivierung und die Aufgabe der Nutzung auf etwas gleich viele Arten aus. Veränderung von Habitaten,

z. B. durch verstärkte Zufuhr von Nährstoffen (Lee & Caporn 1998; Aerts & Bobbink 1999) und die Verhinderung natürlicher Dynamik bedrohen 49% der Arten. Die Bedeutung der letztgenannten Prozesse hat in den vergangenen Jahren zugenommen (Korneck *et al.* 1998).

Prozesse, die die untersuchten Arten spezifisch betreffen sind (i) Umwandlung von Grünland in Äcker nach 1945, Grünland-Eutrophierung, Aufgabe der Grünlandnutzung und Bewaldung von Grünland, (ii) Absenkung des Grundwasserspiegels und Drainage, (iii) Zerstörung kleinräumiger Landschaftselemente (z. B. Wiesenränder, Hecken, Gräben), (iv) intensive Beweidung, (v) Torf- und Sandgewinnung und Bodenverfüllung, und (vi) verminderte Überflutungsdynamik (Korneck *et al.* 1998).

Die große Bedeutung von Landnutzung und Pflege auf die Stadienstruktur der Populationen (**Kapitel 3**), die Dichte der Keimlinge (**Kapitel 3**), die Netto-Reproduktionsrate (**Kapitel 4**) und große Unterschiede zwischen Regionen mit unterschiedlicher Nutzung der untersuchten Flächen (**Kapitel 3, 4, 6**) verdeutlichten, dass deterministische Prozesse die größte Gefährdung für die Lebensfähigkeit und Fortdauer von Populationen der drei Stromtalveilchen darstellen. Verschlechterung der Habitatqualität durch verminderte Nutzung führt zu schrumpfenden Populationen (Wachstumsrate <1), hoher Keimlingsmortalität, und reduzierter Blüten- und Samenbildung (Jensen & Meyer 2001; Jacquemyn *et al.* 2003; Brys *et al.* 2005). Neben diesen deterministischen Prozessen spielt jedoch auch Umweltstochastizität (Bodenfeuchte, Interspezifische Interaktionen und Witterungsbedingungen) eine Rolle vor allem für die Etablierung von Keimlingen und die Reproduktion (**Kapitel 2, 3**). In manchen (Extrem-)Jahren kann die Ausprägung der Umweltbedingungen sogar einen alles überragenden Effekt ausüben (vgl. Morgan 1999). Genetische Stochastizität (d. h. genetische Drift) ist in allen untersuchten Arten und Regionen wirksam (**Kapitel 6**); die Stärke ihres Einflusses variiert zwischen kleinen und großen, isolierten und nicht isolierten Populationen der untersuchten Arten.

Deterministische Prozesse umfassen verminderte Landnutzung oder Nutzungsaufgabe der ehemals extensiv genutzten sub-kontinentalen Stromtalwiesen, Sukzession von offenen Wald- und Gebüschrändern zu geschlossenen reifen Auenwäldern (besonders im Fall von *V. elatior*), Habitatzerstörung und verminderte Störungsfrequenz und –intensität durch ein verändertes Überflutungsregime. Die Akkumulation von Pflanzenstreu, die Ausbreitung von Moosmatten und die Besiedlung durch Sträucher

und Bäume vermindern die Habitatqualität der Auenwiesen. Durch die Etablierung geeigneter Pflegemaßnahmen wie regelmäßige, einmalige Mahd können überlebensfähige Populationen selbst am westlichen Rand des Verbreitungsareals der Arten am Hessischen Oberrhein erhalten werden (**Kapitel 3, 4**). Da es sich bei den untersuchten Arten um ausdauernde Pflanzen handelt, muss jedoch offen bleiben, ob diese Populationen als dauerhaft gesichert gelten können oder sog. Rest-Populationen darstellen (Eriksson 1996). Es konnte im Rahmen der Untersuchungen nicht geklärt werden, ob Habitatzerstörung und –fragmentierung soweit vorangeschritten sind, als dass schon eine Extinktionsspirale angestoßen ist (Tilman *et al.* 1994; Honnay *et al.* 2004). Auf der anderen Seite werden zur Zeit erfolgreiche Maßnahmen unternommen, durch die Übertragung von samenhaltigem Mahdgut aus artenreichen Spenderbeständen auf ehemalige Äcker und artenarme Grünlandbestände aktiv neue Populationen seltener Stromtalwiesenarten zu etablieren (Donath *et al.* 2003, 2004, 2006; Hölzel & Otte 2003).

Schlussfolgerungen (1)

- **Deterministische Prozesse wie Habitatzerstörung, Verminderung der Habitatqualität und Sukzession spielen vermutlich eine große Rolle für die Überlebensfähigkeit von Populationen der untersuchten Arten.**
- **Zusätzlich beeinflusst Umweltstochastizität maßgeblich die Stadienstruktur der Populationen und die Mortalitätsrate von Keimlingen.**
- **Negative Auswirkungen von Habitatfragmentierung (Isolation, Verminderung der Populationsgröße, Randeffekte) können bei den untersuchten Arten anscheinend durch eine geeignete Nutzung und Pflegemaßnahmen ausgeglichen werden.**
- **Es muss jedoch offen bleiben, ob die noch vorhandenen Populationen dieser ausdauernden Arten tatsächlich überlebensfähig sind, oder sich schon in einer Aussterbespirale befinden.**

B. Unterschiede der Populationsdynamik und der genetischen Struktur zwischen zentralen und peripheren Populationen und die Bedeutung von genetischer Drift und Genfluss

‹*Kapitel 3, 4, 6*›

Die Abundanz und Dichte von Individuen und die Häufigkeit von Populationen verhalten sich über das Verbreitungsgebiet einer Art nicht konstant, sondern nehmen zu den Rändern des Areals normalerweise ab (Lawton 1993; Lesica & Allendorf 1995; für eine kritische Übersicht der Belege der '*abundant centre hypothesis*' siehe jedoch Sagarin & Gaines 2002). Obgleich Muster und ökologische Prozesses am Arealrand viel Aufmerksamkeit von Pflanzenökologen (z. B. Carter & Prince 1981; Bengtsson 1993; Lennon *et al.* 1997; Nantel & Gagnon 1999; Kluth & Bruelheide 2005), Naturschützern und Pflanzengenetikern erfahren haben (Safriel *et al.* 1994; Lesica & Allendorf 1995 und Literaturhinweise in diesen Arbeiten; Durka 1999; Lammi *et al.* 1999; Lönn & Prentice 2002; Van Rossum *et al.* 2003), sind explizite Tests der Hypothese zur höheren Abundanz im Zentrum des Areals ambivalent ausgefallen (Sagarin & Gaines 2002 und Literaturhinweise in dieser Arbeit).

Vom Arealzentrum zum Arealrand hin abnehmende Mächtigkeit und Häufigkeit von Populationen könnte durch verminderte Wuchsleistung, verringerte Reproduktion und eingeschränktes Ausbreitungsvermögen in Folge geringerer Habitatqualität am Arealrand bedingt sein (Pigott & Huntley 1981; García *et al.* 2000; Dorken & Eckert 2001). Zusätzlich könnten die für die Keimung nötigen Habitatbedingungen oder Schutzstellen am Arealrand selten sein (Dinsdale *et al.* 2000; Jump & Woodward 2003). Als Folge wären periphere Pflanzenpopulationen (i) stärker isoliert (Lawton 1993; Lesica & Allendorf 1995) und würden (ii) weniger Individuen enthalten als zentrale Populationen (Durka 1999; Lammi *et al.* 1999; siehe jedoch Kluth & Bruelheide 2005). Kleine Populationen sind einem erhöhten Extinktionsrisiko durch die Wirkung von Umweltstochastizität und Katastrophen ausgesetzt (Lande 1993; Menges & Dolan 1998), ihre Vitalität ist darüber hinaus durch die erhöhte Wahrscheinlichkeit für eine Paarung zwischen verwandten Individuen in kleinen Populationen erniedrigt (Menges 1991; Fischer & Matthies 1998). Zusätzlich kann die sexuelle Vermehrung in kleinen und/oder isolierten Pflanzenpopulationen durch eine unzureichende Dichte geeigneter Bestäuber limitiert werden (Jennertsen 1988; Ågren 1996). Dies trägt zusätzlich zu einer

Verminderung der Samenproduktion bei (Morgan 1999). Niedrige Habitatqualität und/oder kleine Populationsgrößen am Arealrand führen zu einer erhöhten Variabilität demographischer Prozesse (Nantel & Gagnon 1999) und folglich zu erhöhten Extinktionsraten. Größere Distanzen zwischen geeigneten Habitaten, an denen die Art ausgestorben ist, und besiedelten Habitaten, die als Diasporenspender dienen könnten, vermindern die Wiederbesiedlungsrate leerer Habitate und erniedrigen den Anteil besiedelter Habitate in der Metapopulation.
Die drei untersuchten Arten zeigen ein gemeinsames kontinentales Verbreitungsmuster mit einem Zentrum des Areals in der gemäßigten Zone Osteuropas und West-Sibiriens. Sie erreichen die Westgrenze ihres Areals in Zentral- und West-Europa (Eckstein *et al.* 2006a). Populationen am Hessischen Oberrhein (Deutschland) repräsentieren daher den Arealrand der Arten, während Populationen in den March-Thaya Auen (Tschechien) näher am Verbreitungszentrum angesiedelt sind.

Auf der Basis von Ergebnissen aus Dauerbeobachtungsflächen wurde in **Kapitel 3** und **4** Stadienstruktur und Demographie der Arten zwischen marginalen und zentralen Populationen verglichen. Im Gegensatz zu den Erwartungen waren Keimlingsdichte (**Kapitel 3**) und Gesamtdichte in marginalen Populationen höher als in zentralen. Ähnliche Ergebnisse wurden von Kluth und Bruelheide (2005) für *Hornungia petraea* dokumentiert, während die Hypothese zur höheren Abundanz im Zentrum des Areals für *Cirsium heterophyllum* bestätigt wurde (Jump & Woodward 2003). Niedrigerer Samenansatz in peripheren Populationen bei letzterer Art führten zu niedrigeren Dichten, ein Zusammenhang, der auch bei anderen Arten beobachtet wurde (z. B. Pigott & Huntley 1981; García *et al.* 2000; Dorken & Eckert 2001). Dagegen waren Unterschiede im Samenansatz von *Hornungia petraea* zwischen Regionen gering, so dass die von Kluth & Bruelheide (2005) beobachteten Unterschiede in der Dichte vermutlich mit der Dynamik der Samenbank zusammen hängen. Bei den hier untersuchten Arten zeigten zentrale Populationen höhere Überlebens- und Reproduktionswahrscheinlichkeiten, während der Samenansatz und die Reproduktionsrate in den marginalen Populationen höher (oder ähnlich groß) waren als in zentralen Populationen (**Kapitel 4**). Dies war sehr wahrscheinlich eine Auswirkung unterschiedlichen Managements durch Mahd (siehe **Kapitel 4**), welches die für die erwarteten pflanzengeographischen Muster verantwortlichen Faktoren ausglich. Die intrinsische Wachstumsrate der Populationen

(λ) war in marginalen Populationen etwas höher, die Unterschiede zwischen Regionen waren jedoch statistisch nicht signifikant (**Kapitel 4**).

In Übereinstimmung mit der Hypothese zur höheren Abundanz im Zentrum des Areals waren marginale Populationen bei allen drei Arten kleiner (niedrigere Individuenzahlen) und bei *V. pumila* and *V. stagnina* auch stärker isoliert (größere Entfernung zur nächsten Population) (**Kapitel 6**). Im Fall von *V. elatior* waren umgekehrt zentrale Populationen stärker isoliert als marginale, was vermutlich das Resultat eines höheren Anteils an Auenwäldern entlang der Thaya im Vergleich zum hessischen Oberrhein ist. In Tschechien befinden sich viele der Populationen innerhalb von Wäldern und entlang von Waldwegen, d. h. in Habitaten in einem fortgeschrittenen Sukzessionsstadium. Folglich sind Unterschiede zwischen Regionen in der Verfügbarkeit geeigneter junger oder mittlerer Sukzessionsstadien vermutlich für die stärkere Isolation von Populationen im zentralen Bereich des Areals verantwortlich. Habitatfragmentierung überlagert daher bei *V. elatior* die Effekte der Pflanzengeographie (zentral-marginal) (**Kapitel 6**).

Populationsgenetische Analysen zeigten, dass die genetische Diversität, gemessen als Gendiversität (Lynch & Milligan 1994) und molekulare Varianz (Fischer & Matthies 1998), die Anzahl der Marker (= auswertbare AFLP-Banden) und der Anteil polymorpher Marker (= AFLP-Banden) in marginalen Populationen von *V. pumila* and *V. stagnina* niedriger waren als in den zentralen Populationen, während bei *V. elatior* keine signifikanten Unterschiede festzustellen waren (**Kapitel 6**).

Periphere Populationen von *Viola elatior* und zentrale Populationen von *V. pumila* und *V. stagnina* waren im Durchschnitt etwa 0,5 km von der nächsten Population derselben Art entfernt, während zentrale Populationen von *V. elatior* und marginale Populationen von *V. pumila* etwas 1 km voneinander getrennt waren. Diese Entfernung ist innerhalb des durchschnittlichen Flugradius von Bienen und Hummeln (Hymenoptera, Apidae) von etwa 2,5 bis 5 km auf der Suche nach Pollen und Nektar (Eckert 1933, Araújo *et al.* 2004). Da die meisten Bienen sich auf Nahrungssuche nur etwa 1 km von ihrem Brutplatz entfernen (Eckert 1933), stellt eine Entfernung von 1 km vermutlich ein Limit für regelmäßigen Genfluss durch Pollen dar (siehe auch Kwak *et al.* 1998 und Zitate in dieser Arbeit). Diese Aussage wird durch unsere Analysen der relativen Bedeutung von Genfluss und genetischer Drift bestätigt (**Kapitel 6**).

Auf der Basis eine Trittstein-Modells der Populationsstruktur, d. h. eines Modells, in welchem die Wahrscheinlichkeit von Genfluss für benachbarte Populationen am größten ist, haben Hutchison und Templeton (1999) einen Ansatz zur Untersuchung der relativen Bedeutung von Genfluss und genetischer Drift vorgeschlagen, der auf einer Analyse der Zusammenhänge zwischen genetischer (F_{ST}) und geographischer Distanzen beruht (siehe **Kapitel 6**). Ein Gleichgewicht zwischen Genfluss und genetischer Drift, d. h. ein Muster von Isolation durch geographische Distanz, wäre hier durch einen monoton positiven Zusammenhang zwischen genetischer und geographischer Distanz charakterisiert. Bedingt durch den homogenisierenden Einfluss von Genfluss sollten Populationen, die nahe beieinander liegen, nicht nur durch geringere genetische Distanzen gekennzeichnet sein, sondern die Variation der genetischen Distanz zwischen Paaren von Populationen sollte auch gering sein. Mit zunehmender geographischer Entfernung nimmt der relative Einfluss des Genflusses ab. Weit voneinander entfernte Populationen sollten daher sowohl genetisch stärker unähnlich sein und die Variation der genetischen Distanz zwischen Paaren von Populationen sollte aufgrund genetischer Zufallsprozesse zunehmen. Falls eine Art jedoch noch nicht lange genug in einem Gebiet vorkommt oder wenn die Ausbreitung der Art begrenzt ist, wird sich noch kein Gleichgewicht zwischen Genfluss und genetischer Drift eingestellt haben. In diesem Fall deutet das Fehlen einer Korrelation zwischen genetischer und geographischer Distanz in Kombination mit relativ geringer Variation von F_{ST} auf eine relative größere Bedeutung von Genfluss gegenüber genetischer Drift (Hutchison & Templeton 1999). Falls der Genfluss relativ groß bleibt, wird sich dieses Muster erhalten. Wenn jedoch die Umweltbedingungen zu einer Habitatfragmentierung und verstärkter Isolation von Populationen innerhalb der Region führen, wird sich der Einfluss genetischer Drift verstärken und die Variationsbreite von F_{ST} zunehmen.

Die Ergebnisse der Untersuchungen zeigten, dass in Anlehnung an den Ansatz von Hutchison and Templeton (1999) bei den drei Stromtalveilchen kein Gleichgewicht zwischen Genfluss und genetischer Drift festzustellen war. Das Muster von genetischer und geographischer Distanz deutete auf einen stärkeren Einfluss von Genfluss bei allen Art-Region Kombinationen mit einer durchschnittlichen Entfernung zur nächsten Population von 0,5 km oder weniger. Der Einfluss von genetischer Drift überwog den von Genfluss bei Art-Region Kombinationen, in denen Populationen mehr als 1 km von der nächsten Population entfernt waren und besonders im Fall von peripheren

Populationen von *V. stagnina*, die im Durchschnitt 5 km voneinander entfernt lagen. Stärkere genetische Divergenz zwischen marginalen Populationen von *V. pumila* und *V. stagnina* (Φ_{ST}-Werte) bestätigten diese Folgerungen.

In ähnlicher Weise fanden Culley und Grubb (2003) einen relativ größeren Einfluss von genetischer Drift auf die Populationsstruktur fragmentierter Populationen von *Viola pubescens* (Spanne der paarweisen geographischen Distanzen von 0.3 bis 45 km). Eine Tendenz zu größerer Divergenz zwischen marginalen als zwischen zentralen Populationen wurde auch bei *Silene nutans* gefunden (Van Rossum *et al.* 2003). Ähnlich starke genetische Differenzierung zwischen Populationen wie in **Kapitel 6** wurden bei anderen kleistogamen (Auge *et al.* 2001; Culley & Grubb 2003), selbstbestäubten (Durka 1999) oder seltenen Pflanzenarten dokumentiert (Travis *et al.* 1996; Lammi *et al.* 1999; Schmidt & Jensen 2000). Große genetische Divergenz wurde auch zwischen Populationen dreier seltener Arten der Gattungen *Silene* festgestellt (Dolan 1994; Lammi *et al.* 1999; Van Rossum *et al.* 2003), während die Populationsstruktur der gemeinen und weit verbreiteten Art *Silene flos-cuculi* durch hohen Raten von Genfluss gekennzeichnet war (Galeuchet *et al.* 2005).

Anders als in vielen anderen Studien (z. B. Dolan 1994; Travis *et al.* 1996; Fischer & Matthies 1998; Lammi *et al.* 1999; Culley & Grubb 2003) war bei den hier untersuchten Arten die genetische Diversität der Populationen nicht mit der Populationsgröße oder der Isolation der Populationen korreliert. Zusammenhänge zwischen aktueller Populationsgröße und molekularer Varianz können durch die Geschichte der Populationen (Alter, Gründereffekte), die räumliche Konfiguration (Isolation, Anzahl und Distanz zu anderen Populationen) oder Zufallseffekte verschleiert werden (Oostermeijer *et al.* 1994b; Schmidt & Jensen 2000).

Periphere Populationen von *V. elatior*, *V. pumila*, und *V. stagnina* hatten jeweils einen, zwei und acht seltene lokale genetische Marker im Vergleich mit zentralen Populationen verloren. Zusätzlich fehlten in peripheren Populationen von *Viola stagnina* ein seltener aber weit verbreiteter Marker und drei häufige, weit verbreitete Marker, die in ≥40% der zentralen Populationen vorkamen und eine relative Häufigkeit von ≥0.1120 hatten. Der zusätzliche Verlust seltener lokaler genetischer Marker in peripheren Populationen verdeutlichte den Einfluss zufälliger genetischer Drift besonders bei *V. stagnina*.

Gleichzeitig enthielten periphere Populationen jedoch einige wenige genetische Marker, die in zentralen Populationen fehlten. So wurde ein häufiger, weit verbreiteter Marker

bei *V. elatior*, einer bei *V. pumila* und ein häufiger, lokaler Marker bei *V. stagnina* gefunden. Dieser Beitrag zur gesamten genetischen Diversität der Arten macht einen Teil des potentiellen Werts marginaler Populationen unter Artenschutzgesichtspunkten aus (Safriel *et al.* 1994; Lesica & Allendorf 1995; Durka 1999). Obgleich ihr taxonomischer Status noch nicht abschließend geklärt ist, deutet das Vorkommen der Varietät *lactaeoides* von *V. stagnina* in den Niederlanden (Weeda 2001) darauf hin, dass räumliche Isolation und genetische Divergenz die Abspaltung neuer Taxa und die Artbildung am Rande des Verbreitungsareals fördern kann (Lesica & Allendorf 1995).

S c h l u s s f o l g e r u n g e n (2)

- **Die Vorhersagen der Hypothese zur höheren Abundanz im Zentrum des Areals konnten für die untersuchten Arten nur teilweise bestätigt werden: Marginale Populationen waren durchweg kleiner als zentrale Populationen. Dagegen war die Dichte von Keimlingen und die Gesamtdichte der Populationen in marginalen Populationen höher.**
- **Eine stärkere Isolation und geringere genetische Diversität wurden in marginale Populationen von *V. pumila* und *V. stagnina* gefunden.**
- **Im Fall von *V. elatior* überlagerten Effekte von Habitatfragmentierung in Folge unterschiedlicher Landschaftsstruktur in marginalen und zentralen Populationen das erwartete pflanzengeographische Muster und führten zu geringerer Isolation marginaler Populationen. Genetische Diversität unterschied sich nicht zwischen den untersuchten Regionen.**
- **Genfluss hatte in allen Art-Region Kombinationen mit einer durchschnittlichen Entfernung von <0,5 km einen relative größeren Einfluss auf die Populationsstruktur als genetische Drift (d. h. *genetische Zufallsprozesse*), während die Bedeutung genetischer Drift überwog, wenn die durchschnittliche Distanz zur nächsten Population >1 km war.**
- **Bei den untersuchten Arten konnte kein Gleichgewicht zwischen Genfluss und genetischer Drift festgestellt werden. Die genetische Diversität der Populationen war nicht signifikant mit der Populationsgröße oder dem Grad der Isolation korreliert.**

C. Bedeutung von Bestäubungssystem und Pollenquelle für die Samenproduktion und die Fitness der Nachkommen

‹Kapitel 5›

Viele früher weiter verbreitete Pflanzenarten sind heute in Folge von Landnutzungsänderungen, Habitatzerstörung oder Fragmentierung auf kleine und isolierte Populationen beschränkt (z. B. Saunders *et al.* 1991; Jensen & Schrautzer 1999; Lienert *et al.* 2002; Eriksson *et al.* 2002; Honnay *et al.* 2004). Kleine und isolierte Populationen sind einem erhöhten Aussterberisiko durch Umweltstochastizität oder Katastrophen ausgesetzt (Lande 1993; Fischer & Stöcklin 1997; Matthies *et al.* 2004). Ihre Lebensfähigkeit kann potentiell jedoch auch durch eine erhöhte Wahrscheinlichkeit der Kreuzung zwischen eng verwandten Individuen in kleinen Populationen beeinträchtigt werden. Generell kann der bei der Bestäubung einer Blüte wirksam werdende Pollen von den Staubblättern derselben Blüte (autogame Selbstung), von einer anderen Blüte desselben Sprosses oder desselben genetischen Individuums (geitonogame Selbstung) oder von einem Spross eines anderen genetischen Individuums stammen (Xenogamie, Auskreuzung) (Richards 1997). Daher ist vor allem bei kleinen und/oder isolierten Populationen die Wahrscheinlichkeit für Selbstbestäubung erhöht (Barrett & Kohn 1991; Dudash & Fenster 2000).

Selbstbestäubung wiederum kann zu sog. Inzuchtdepression führen (z. B. Husband & Schemske 1996; Culley 2000; Dudash & Fenster 2000; Charlesworth 2003), d. h. zu im Vergleich mit ausgekreuzten Nachkommen verminderter Lebensfähigkeit und Fitness (z. B. Waller 1984; Mitchell-Olds & Waller 1985; Dudash 1990), die sich in erhöhter Abortneigung und einer Reduktion des Samenansatzes, der Keimung, der Fitness der Nachkommen sowie Veränderungen der Populationsstruktur äußern (Waller 1984; Dudash 1990; Fischer & Matthies 1997; Richards 1997; Berg & Redbo-Torstensson 1999; Culley 2000; Karrenberg & Jensen 2000; Lienert & Fischer 2002).

Während die meisten offenen Blüten selbst-kompatibler Arten potentiell fremd- oder selbstbestäubt sein können, stellen kleistogame (CL) Blüten, die obligat selbstbestäubt sind, einen extremen Fall der Blütenreduktion dar (Richards 1997). Kleistogamie ist von mindestens 256 Arten aus 56 Blütenpflanzen-Familien bekannt (Lord 1981). Bei „echten“ kleistogamen Arten (Plitmann 1995) können sowohl offene, potentiell fremdbestäubte Blüten (chasmogame Blüten, CH) als auch CL Blüten an derselben Pflanze ausgebildet sein. Das CH-CL System stellt ein gemischtes Bestäubungssystem dar, das sowohl zu

potentiell variablem (CH) als auch zu relativ invariablem Nachwuchs (CL) führt. Es gibt jedoch nur wenige Studien, in denen explizit zwischen Bestäubungsmodus und Blütentyp unterschieden wird (z. B. Culley 2000), während in den meisten Fällen angenommen wird, dass CH Blüten fremdbestäubt sind.

Die hier untersuchten Arten sind charakteristische Elemente artenreicher Stromtalwiesen und nährstoffarmer Feuchtwiesen der Verbände *Cnidion* und *Molinion*. Die Wiederansiedlung dieser Vegetationstypen auf ehemaligen Ackerflächen durch die Aufbringung diasporenhaltigen Mahdguts aus artenreichen Spenderbeständen ist das Ziel umfangreicher Naturschutzprojekte (Donath *et al.* 2003; Hölzel & Otte 2003). Da der Auftrag von Mahdgut zur Etablierung von neuen, anfangs kleinen Populationen aus Individuen verschiedener Spenderpopulationen führen könnte, war ein Ziel von **Kapitel 5** die möglichen Konsequenzen verschiedener Pollenquellen und die Auswirkungen des Blütentyps auf Samenproduktion, Sameneigenschaften und die Fitness der Nachkommen der Arten in zwei unterschiedlichen experimentellen Umgebungen zu untersuchen. Die folgenden Experimente wurden durchgeführt:

- **Experiment I**: Ein Vergleich von Pflanzen, deren Bestäubung durch Insekten mit Hilfe perforierter Zellophantüten verhindert wurde, mit einer unbehandelten Kontrollgruppe diente der Analyse der Effekte von Selbstbestäubung und potentieller Fremdbestäubung auf die Produktion von CH Kapseln und den CH Samenansatz.
- **Experiment II**: Um die Auswirkungen der Pollenquelle auf Samenanzahl und Samenmasse zu untersuchen, wurden Einzelblüten entweder (i) mit Pollen derselben Blüte (Selbstbestäubung), (ii) einer anderen Pflanze derselben Population (potentielle Fremdbestäubung) oder (iii) einer anderen Pflanze aus einer anderen Population bestäubt (Fremdbestäubung). Zusätzlich (iv) wurden Samen aus CL Kapseln in der Analyse berücksichtigt.
- **Experiment III**: Um die Auswirkungen der Pollenquelle auf die Fitness der Nachkommen unter verschiedenen Umweltbedingungen zu testen, wurden zwei experimentelle Umgebungen geschaffen. Dazu wurde nach dem Ende von Experiment I die in den Töpfen verwendete Erde getrennt für jede Art gesammelt. Die Hälfte des Bodens wurde hitzesterilisiert (115° C, 24 h), die andere Hälfte wurde nicht behandelt. Danach wurde entweder sterilisierte oder nicht-sterilisierte Erde neuen, mit Pflanzerde gefüllten Töpfen zugegeben. Keimlinge der untersuchten Arten, die aus Samen nach Bestäubung aus unterschiedlichen Pollenquellen hervorgegangen waren (Experiment II), wurden dann einzeln in diese vorbereiteten Töpfe gepflanzt.

Die Entwicklung und Reifung von CH und CL Kapseln verlief bei den untersuchten Arten zeitlich getrennt, wobei CL Kapseln erst nach dem Verblühen der CH Blüten gebildet wurden. Es ist nicht unwahrscheinlich, dass die Entwicklung dieser beiden

Blütentypen durch unterschiedliche Umweltreize ausgelöst wird (Le Corff 1993), und dass bei Arten mit nicht überlappender Entwicklung der Blütentypen die Produktion der CL Kapseln je nach reproduktivem Erfolg der CH Blüten reguliert wird (Redbo-Torstensson & Berg 1995).

Bei den hier untersuchten Veilchenarten hatte der Ausschluss von Bestäubern nur geringe Auswirkungen auf die Ausbildung von CH Kapseln und Samen. Bis zu 90% der CH Kapseln kamen bei *V. elatior* und *V. pumila* gar nicht zur Reife, während CL Kapseln reichlich reife Samen produzierten. Es ist nicht unwahrscheinlich, dass Samen aus CH Kapseln nur einen relativ geringen Beitrag zur gesamten Samenproduktion leisten, aber eventuell in gewissen Jahren eine größere Rolle spielen. Die Tatsache, dass das aus Freilandaufsammlungen ermittelte Samengewicht von *V. pumila* (Hölzel & Otte 2004b) exakt dem Wert von Samen aus CL Kapseln der vorliegenden Arbeit entsprach (1.08 mg in beiden Fällen) kann als Hinweis gewertet werden, dass die Mehrzahl der Samen in natürlichen Populationen möglicherweise aus CL Kapseln stammt.

In beiden oben genannten Arten war das Wachstum der Keimlinge unter „Heimat"-Bedingungen signifikant geringer als unter Kontrollbedingungen (**Kapitel 5**). Dies ist sehr wahrscheinlich auf die negativen Auswirkungen von Bodenmikroorganismen zurückzuführen (Vorkultureffekt), der auch schon für viele andere wildwachsende Pflanzenarten dokumentiert wurde (Bever 1994; Packer & Clay 2000; Klironomos 2002). Obgleich wir keine generellen Wechselwirkungen zwischen Pollenquelle und der vorgenommenen Bodenvorbehandlung nachweisen konnten, deuten unsere Ergebnisse trotzdem darauf hin, dass die relative Fitness geselbsteter Nachkommen von *V. stagnina* unter Stressbedingungen (d. h. mit der mikrobiellen Umgebung der Mutterpflanze) signifikant höher war, als die ausgekreuzter Nachkommen Die Rekombination von Individuen weit voneinander entfernter Populationen erzeugte Nachkommen, die Zeichen von Auskreuzungsdepression unter Stressbedingungen zeigten.

In Übereinstimmung mit unseren Ergebnissen (**Kapitel 5**), unterschied sich in einer großen Population von *Pedicularis palustris* die Anzahl der Samen pro Kapsel nicht zwischen Blüten, die mit unterschiedlichen Pollenquellen bestäubt wurden; autogame Selbstung führte in einer kleinen Population jedoch zu reduzierter Samenbildung (Karrenberg & Jensen 2000). In drei weiteren Europäischen Veilchenarten wurden keine Unterschiede bezüglich der Degeneration von Samen zwischen CH und CL Kapseln

gefunden (Berg & Redbo-Torstensson 1999), jedoch ist nicht eindeutig, ob die CH Blüten in dieser Studie tatsächlich ausschließlich fremdbestäubt waren.

Im Hinblick auf die Biomasse von Keimlingen nach acht Wochen zeigten die untersuchten Arten keine Hinweise auf Inzuchtdepression (**Kapitel 5**). Bei *V. stagnina* unterschied sich die relative Fitness zwischen den Bodenbehandlungen, aber im Durchschnitt gab es keine Unterschiede zwischen Pollenquellen oder Blütentypen. Auch Berg & Redbo-Torstensson (1999) fanden keine Inzuchtdepression bei drei anderen Arten der Gattung *Viola*. Dies könnte ein Hinweis darauf sein, dass mögliche Allele, die im homozygoten Zustand in Folge von Selbstung negative Effekte zeitigen, im Zuge der Phylogenie dieser selbstbestäubten Taxa eliminiert wurden. In ähnlicher Weise variierte auch bei *Viola canadensis* die relative Fitness von geselbsteten und ausgekreuzten Nachkommen zwischen den untersuchten Merkmalen, aber der Grad der Inzuchtdepression oder Unterschiede zwischen den Blütentypen waren generell gering (Culley 2000).

Die Tatsache, dass bei *V. stagnina* Nachkommen von Pflanzen, die mit dem Pollen einer anderen Population bestäubt wurden, unter „Heimat"-Bedingungen eine signifikant geringere Fitness aufwiesen als Pflanzen, die mit Pollen derselben Population bestäubt wurden, ist ein Hinweis auf Auskreuzungsdepression (**Kapitel 5**). Höhere Fitness von Kreuzungen innerhalb von Populationen im Vergleich zu Kreuzungen zwischen Populationen wurde auch für *Gentianella germanica* dokumentiert (Fischer & Matthies 1997).

Aufgrund der starken Isolation von Populationen von *V. stagnina* im Untersuchungsgebiet (**Kapitel 3, 6**) erfolgt nur ein relativ geringer Genfluss und die Differenzierung zwischen Populationen ist hoch (**Kapitel 6**). Daher können Kreuzungen zwischen diesen isolierten Populationen möglicherweise zu biochemischen oder physiologischen Inkompatibilitäten zwischen Genen oder dem Bruch koadaptierter Genkomplexe führen (Dobzhansky 1972; Siikamäki 1999) und damit verbunden zu verminderter Fitness der Nachkommen speziell unter Stressbedingungen. Auskreuzungsdepression ist unter anderem in der Artenschutzbiologie von Belang, wenn es um Anstrengungen geht, die genetische Diversität und Heterozygotie seltener Pflanzenarten zu erhalten oder wieder herzustellen (Dudash & Fenster 2000; Fenster & Galloway 2000; Montalvo & Ellstrand 2001; Edmands & Timmermann 2003).

Die Ergebnisse unserer Untersuchungen legen nahe, dass die Schaffung kleiner Gründerpopulationen durch die Übertragung diasporenhaltigen Mahdguts im Rahmen von Artenschutzprojekten, die mit einer erhöhten Wahrscheinlich für Selbstbestäubung verknüpft ist, vermutlich keine direkte Gefahr für die Lebensfähigkeit dieser seltener Arten darstellt. In neu geschaffenen Populationen, die aus Genotypen unterschiedlicher Ausgangspopulationen entstanden sind, besteht jedoch die Gefahr, dass Keimlingswachstum und Lebensfähigkeit der Population durch Auskreuzungsdepression in Folge von Fremdbestäubung beeinträchtigt werden. Daher sollte das Mischen von Mahdgut verschiedener Populationen wenn möglich vermieden werden.

S c h l u s s f o l g e r u n g e n (3)

- **Die untersuchten Arten sind vermutlich nicht pollenlimitiert, da der Ausschluss von Bestäubern nur geringe Auswirkungen auf die Produktion von CH Kapseln hatte. Im Allgemeinen produzierten die Arten sehr viel mehr CL als CH Kapseln.**
- **Samengewicht und –anzahl unterschieden sich nicht signifikant nach Bestäubung aus verschiedenen Pollenquellen. CL Samen waren jedoch signifikant leichter als CH Samen.**
- **Das Keimlingswachstum unter „Heimat"-Bedingungen, d. h. auf Boden, der mit Vorkulturerde derselben Art beimpft wurde, war deutlich gegenüber dem Wachstum unter Kontrollbedingungen reduziert.**
- **Bei *V. stagnina* war unter „Heimat"-Bedingungen die relative Fitness von geselbsteten Keimlingen größer als die von ausgekreuzten Nachkommen. Hohe genetische Divergenz zwischen Populationen in Folge starker Isolation kann für die beobachtete Auskreuzungsdepression dieser Art verantwortlich sein.**
- **Es gibt keine Hinweise auf Inzuchtdepression bei den untersuchten Arten.**
- **Die Schaffung anfangs kleiner Gründungspopulationen durch die Übertragung diasporenhaltigen Mahdguts im Rahmen von Artenschutzprojekten stellt vermutlich keine direkte Gefahr für die Lebensfähigkeit dieser seltenen Arten dar. Das Mischen von Mahdgut aus voneinander entfernten Populationen sollte jedoch vermieden werden, um mögliche Auskreuzungsdepression zu verhindern.**

D. Identifizierung sensibler Stadien im Lebenszyklus der untersuchten Arten zur Entwicklung von Maßnahmen zu ihrem Schutz

‹Kapitel 4›

Das Wachstum und die Lebensfähigkeit von Populationen hängen letztlich von den demographischen Raten (engl. *vital rates*) der Individuen, d. h. *Überleben*, *Wachstum* und *Fruchtbarkeit* (*Reproduktion*) ab (siehe Caswell 2001). Umweltbedingungen (abiotische Bedingungen und biotische Interaktionen) üben Selektionskräfte aus, die zur Entwicklung und Abfolge spezifischer Merkmalskombinationen (z. B. Chapin *et al.* 1993) in bestimmten Habitattypen, entlang von Sukzessionsserien oder räumlichen Gradienten vom Zentrum zur Peripherie des Verbreitungsareals führen können (Sagarin & Gaines 2002). Da das Schicksal von Organismen von ihrem Alter oder Entwicklungsstadium abhängt, kann ein demographischer Artenschutzansatz, d. h. die populationsbiologische Analyse verschiedener Aspekte des Lebenszyklus von Organismen (Caswell 2001), wichtige und notwendige Erkenntnisse für den Schutz und die Erhaltung seltener und gefährdeter Arten liefern (z. B. Crouse *et al.* 1987; Caswell & Brault 1992; Schemske *et al.* 1994; Menges 2000; Pfab & Witkowski 2000; Caswell 2001; Morris & Doak 2002; Nicolè *et al.* 2005). Die Identifizierung sensibler Stadien im Lebenszyklus bedrohter Arten steht dabei im Mittelpunkt der Überlegungen in der Artenschutzbiologie (Caswell 2000, 2001; Morris & Doak 2002). Ein klassisches Beispiel ist die Untersuchung von Crouse *et al.* (1987) über den Lebenszyklus der Unechten Karettschildkröte (*Caretta caretta*). Die Ergebnisse dieser Studie auf der Grundlage populationsbiologischer Modelle und Elastizitätsanalysen lenkten den Fokus der Artenschutzbemühungen, der sich traditionell auf den Schutz der Gelege der Art an den Stränden beschränkte, auf einen effektiven Schutz von juvenilen und adulten Tieren im Meer vor dem Tod in Fischnetzen. Die Einführung von Einrichtungen zur Verhinderung des Beifangs von Schildkröten auf Krabbenkuttern im Jahre 1987 wurde durch diese Untersuchungen angestoßen (Caswell & Brault 1992).

Generell gibt es zwei Arten von Analysen zur Ermittlung des Einflusses einzelner Lebenszyklusstadien auf das Populationswachstum (Horvitz *et al.* 1997; Caswell 2000, 2001): (i) *prospektive* Analysen, d. h. Sensitivität und Elastizität, und (ii) *retrospektive* Analysen, d. h. Lebenstafel-Reaktionsexperimente (LTRE, engl. *life table response experiments*).

Prospektive Analysen schauen nach vorne und untersuchen die Auswirkungen von Variabilität demographischer Raten auf die intrinsische Populationswachstumsrate (λ). Sie sind nicht an die tatsächlich beobachtete Variabilität demographischer Raten gebunden, sondern können z. B. auch die Auswirkungen eigentlich unmöglicher Veränderungen des Lebenszyklus quantifizieren (z. B. '*Was würde passieren, wenn Schweine Flügel hätten?*', Horvitz *et al.* 1997; Caswell 2000). Prospektive Analysen hängen weder von der Kenntnis der Variabilität demographischer Raten in der Vergangenheit, Gegenwart oder Zukunft ab, noch ist die Kenntnis dieser Variabilität für die Analyse hilfreich. *Sensitivität* ($\mathbf{s}_{ij}$) entspricht der Steigung von λ in Abhängigkeit von (kleinen) absoluten Änderungen einzelner Zellen der populationsbiologischen Übergangsmatrix (a_{ij}), wobei alle anderen Matrixeinträge konstant gehalten werden (zur Formel der Sensitivität siehe Caswell 2001, S. 209). Da populationsbiologische Matrizen sowohl Übergangswahrscheinlichkeiten enthalten, die zwischen 0 und 1 rangieren, als auch Werte für die Fruchtbarkeit (Anzahl von Nachkommen pro Mutterpflanze), die sich oft in einer anderen Größenordnung bewegen, kann es auch sinnvoll sein, die Reaktion von λ auf *proportionale* statt auf *absolute* Veränderungen von Matrixzellen zu untersuchen. Diese wird mit Hilfe der *Elastizität* ($\mathbf{e}_{ij}$) analysiert, die der Steigung von *log* λ in Abhängigkeit von *log* a_{ij} entspricht (de Kroon *et al.* 1986; Caswell 2001). Elastizität und Sensitivität sind über die Formel $\mathbf{e}_{ij} = \mathbf{s}_{ij} \times (a_{ij} / \lambda)$ miteinander verbunden. Über eine demographische Matrix addieren sich die Werte der Elastizität zu eins.

Retrospektive Analysen schauen zurück und beschäftigen sich mit der Frage, welchen Einfluss tatsächlich beobachtete Unterschiede in demographischen Raten auf beobachtete Unterschiede in λ haben (Caswell 2000). Da diese Analysen die *Variabilität* von λ als Funktion der *Variabilität* von demographischen Raten betrachten, ist die Kenntnis dieser tatsächlichen Variabilität als solche essentiell. Das LTRE hat einige Gemeinsamkeiten mit der Varianzanalyse (ANOVA), da es auch auf linearen Modellen von λ als Funktion von 'experimentellen' Behandlungen basiert, auf der Aufspaltung von Effekten beruht und die Analyse multi-faktorieller Designs (mit festen oder zufälligen Faktoren) ermöglicht. Anders als eine ANOVA beschränkt sich das LTRE jedoch nicht auf den Nachweis statistisch signifikanter Unterschiede von λ oder der demographischen Raten. Vielmehr können selbst winzige Differenzen zwischen zwei Wachstumsraten auf die ihnen zugrunde liegenden Unterschiede der demographischen Raten hin untersucht werden (siehe Beispiel in Caswell 1996). Bei einem LTRE wird der

Beitrag eines Lebenszyklusübergangs ($\mathbf{c}_{ij}$) zur beobachteten Variabilität von λ aus dem Produkt der Differenz des entsprechenden Matrixelements zwischen den verglichenen Matrizen (Δa_{ij}) und der Sensitivität von λ gegenüber diesem Element ($\mathbf{s}_{ij}$) (geschätzt an einer gemittelten Matrix, Caswell 1996, 2001) ermittelt. Daher tragen Matrixübergänge, die in den verglichenen Matrizen nicht variieren, oder die einen geringen Effekt auf λ ausüben, auch nichts oder nur wenig zur beobachteten Differenz von λ bei.

In Untersuchungen zum Artenschutz wird statt Δa_{ij} oft die Varianz oder Standardabweichung von a_{ij} in einer Durchschnittsmatrix der Art verwendet (z. B. Ehrlén & van Groenendael 1998). Caswell (2000, 2001) bestreitet jedoch, dass retrospektive Analysen zur Identifizierung sensibler Stadien im Lebenszyklus verwendet werden können, denn Matrixelemente (a_{ij} d. h. Übergangswahrscheinlichkeiten oder Fertilität), können einen geringen Beitrag zur beobachteten Differenz von λ leisten, weil entweder die Variation von a_{ij} physiologisch bzw. entwicklungsbiologisch beschränkt ist (Caswell 2001) oder andere Faktoren wie z. B. die Umwelt eine Rolle spielen. Im ersten Fall wäre a_{ij} ein schlechtes Ziel von Managementmaßnahmen, während Management im zweiten Fall die Variation von a_{ij} positiv beeinflussen könnte. Nur prospektive Analysen, die funktionale Zusammenhänge zwischen Übergängen und Populationswachstum untersuchen, können die demographischen Raten mit dem größten Einfluss auf das Populationswachstum identifizieren. Ob diese demographischen Raten dann mit Hilfe von Managementmaßnahmen verändert werden können, hängt vom Lebenszyklus und der Biologie der betrachteten Art ab. Retrospektive Analysen können in der Artenschutzbiologie jedoch verwendet werden, um die Gründe für den Rückgang von Arten zu erforschen. Caswell (2001) unterscheidet vier Hauptaufgaben des demographischen Artenschutzes, die die Verwendung unterschiedlicher demographischer Verfahren erfordern (Tabelle 8.1).

Tabelle 8.1 Aufgaben und Methoden demographischer Analysen im Artenschutz (Caswell 2001).

Aufgabe	**Methode**
Einschätzung des Status von Populationen	Ermittlung von λ und seiner Messunsicherheit
Diagnose von Gründen für den Rückgang von Populationen	Retrospektive Analysen (LTRE)
Entwicklung von Managementstrategien	Prospektive Analysen (Elastizität, Sensitivität)
Prognose der Populationsentwicklung	Populations-Projektionen

Die untersuchten Arten gehören zu einer Gruppe von europaweit gefährdeten Arten, die besonderer Artenschutzmaßnahmen bedürfen, und stehen in vielen Europäischen Ländern auf den Roten Listen (z. B. Korneck *et al.* 1996; Schnittler & Günther 1999). Die Analyse von Verbreitungsdaten zeigt, dass die Vorkommen der Arten in den letzten Jahrzehnten einen ernsten Rückgang um etwa 50% erlebt haben (Eckstein *et al.* 2006a), der zu einer starken Fragmentierung und Isolation der Populationen führte (**Kapitel 3, 6**). Damit gehören sie zur Gruppe der „Neuen Seltenen" (engl. *'new rares'*, Oostermeijer 1996), die empfindlicher gegenüber Isolation und Arealschrumpfung sind als „natürlicherweise" seltene Pflanzen (Huenneke 1991). Das Ziel der in **Kapitel 4** vorgestellten Untersuchung war daher, zu versuchen, sensible Stadien im Lebenszyklus der Arten zu identifizieren und mögliche Bedrohungen für die Lebensfähigkeit der Populationen und ihren Schutz abzuschätzen.

Die Analyse gründete auf der Erfassung des Schicksals markierter Individuen in 27 Populationen in Auenwiesen entlang der Thaya (Tschechien) und des Oberrheins (Deutschland). Die Information aus drei bis sechs Populationen pro Art und Region wurden gepoolt, um robuste, auf fünf Lebenszyklusstadien basierende Levkovitch Übergangsmatrizen zu erhalten. Mit Hilfe demographischer Matrixberechnungen (siehe Caswell 2001) erhielten wir die Elastizität der Populationswachstumsrate gegenüber einzelnen Matrixübergängen, demographischen Raten und Lebenszyklusstadien. Für die Analyse von Unterschieden der Populationswachstumsrate zwischen Arten und Regionen wurde ein zweifaktorielles LTRE mit festen Effekten berechnet.

Die Ergebnisse der Elastizitätsanalyse zeigten, dass trotz einiger Unterschiede zwischen Regionen bei *V. elatior* hauptsächlich kleine vegetative, sowie kleine und große blühende Pflanzen den größten Einfluss auf λ hatten, während bei *V. pumila* der Einfluss aller vier adulten Stadien auf λ etwa gleich groß war (**Kapitel 4**). Diese beiden Arten zeigten auch ähnliche Elastizitäten in Bezug auf die demographischen Raten (Tabelle 8.2). Im Gegensatz dazu schwankte der Einfluss verschiedener Stadien und demographischer Raten bei *V. stagnina* stark zwischen den Untersuchungsregionen. Populationen dieser Art von der Thaya zeigten die höchste Elastizität gegenüber der Überlebensrate, während Populationen am Rhein die höchste Elastizität aller Art-Region Kombinationen gegenüber der Fruchtbarkeit und die niedrigste gegenüber der Überlebensrate hatten. Generell lagen die Elastizitäten der untersuchten Arten gegenüber den demographischen Raten innerhalb der Spanne, die für polykarpe, ausdauernde Arten

offener Habitate typisch ist (Silvertown *et al.* 1993). Die beobachtete große Variation der Elastizität zwischen Regionen bei *V. stagnina* ist vermutlich auch auf eine große Variation von λ zurückzuführen, denn Elastizität und λ können nicht unabhängig voneinander betrachtet werden.

Tabelle 8.2. Elastizität (**e**) des Populationswachstums von *Viola elatior*, *V. pumila* und *V. stagnina* an Thaya und Rhein gegenüber den demographischen Raten *Überleben*, *Wachstum* und *Fruchtbarkeit*.

Art	Region	**e** (Überleben)	**e** (Wachstum)	**e** (Fruchtbarkeit)
V. elatior	Thaya	0.4546	0.4128	0.1326
	Rhein	0.4672	0.4054	0.1274
V. pumila	Thaya	0.5366	0.3669	0.0965
	Rhein	0.4648	0.4312	0.1040
V. stagnina	Thaya	0.6527	0.3355	0.0119
	Rhein	0.3157	0.4845	0.1998

Dass Populationen mit positiver Wachstumsrate tendenziell eine höhere Elastizität gegenüber Wachstum und Fruchtbarkeit zeigen, wurde schon für *Gentiana pneumonanthe* (Oostermeijer *et al.* 1996), sowie für *Cirsium vulgare* und *Pedicularis furbishiae* (Silvertown *et al.* 1996) gezeigt. Die Elastizität gegenüber demographischen Raten variierte über die Zeit (d. h. zwischen den untersuchten Jahren) und zwischen Populationen bei *Agrimonia eupatoria* und *Geum rivale* (Kiviniemi 2002), *Primula vulgaris* (Valverde & Silvertown 1998) und *Heracleum mantegazzianum* (Hüls 2005; Hüls *et al.* 2007), während sie sich bei drei Arten der Gattung *Pinguicula* relativ konstant verhielt (Svensson *et al.* 1993). Die Tatsache, dass Matrixübergänge nicht voneinander unabhängig sind und dass Elastizität mit der Populationswachstumsrate variiert (Oostermeijer *et al.* 1996; Silvertown *et al.* 1996; Caswell 2001), macht die Identifizierung eindeutiger Stadien oder demographischer Raten und Prozesse für Artenschutz und Management bedrohter Arten schwierig. Zumindest sollten die Ergebnisse von Elastizitätsanalysen vorsichtig interpretiert werden. Zusätzlich erhalten durch die Berechnung der Elastizität als Produkt von Sensitivität und dem Quotient von Übergangswahrscheinlichkeit (a_{ij}) und λ (de Kroon *et al.* 1986; Caswell 2001) häufige Übergänge oft eine höhere Elastizität, während seltene Übergänge wahrscheinlich oft bessere Angriffspunkte für Maßnahmen des Artenschutzes und der Artenerhaltung wären.

Zum Beispiel war die Fruchtbarkeit von Populationen von *V. stagnina* an der Thaya bedingt durch niedrige Habitatqualität in Folge fehlender oder unregelmäßiger Nutzung der Wiesen schon sehr niedrig (siehe **Kapitel 3, 4**), was wiederum zu Elastizitäten gegenüber der Fruchtbarkeit von nahe Null führte. Dies könnte man nun so interpretieren, dass diese demographische Rate (und das Keimlingsstadium) nicht signifikant zu λ beitragen. Auf der anderen Seite werden diese Populationen vermutlich so lange kein positives Populationswachstum zeigen, bis die Bedingungen für Reproduktion und Keimung durch Veränderungen des Managements und des Störungsregimes soweit verbessert werden, dass erfolgreiche Keimlingsetablierung aus der aktuellen Samenproduktion oder der ausdauernden Samenbank möglich ist.

Eine persistente Samenbank, d. h. ein Reservoir dormanter Samen im Boden, das über mehrere Keimungsperioden lebensfähig bleibt, ist ein typisches Merkmal vieler Pflanzenarten, darunter Vertreter unterschiedlichster Lebenszyklen, Habitate und geographischer Regionen (Leck *et al.* 1989; Baskin & Baskin 2001). Theoretische Modelle haben eine ganze Reihe möglicher Effekte einer Samenbank auf die Demographie, die Populationsstruktur und die Evolutionsbiologie von Pflanzen identifiziert: Vor dem Hintergrund großer zeitlicher und räumlicher Variation klimatischer Bedingungen und der Intensität biotischer Interaktionen stellt eine persistente Samenbank eine Möglichkeit dar, ungeeigneten Umweltbedingungen zu entgehen. Die Samenbank puffert so die Wachstumsrate der Population gegenüber Umweltstochastizität besonders in dynamischen Habitaten oder bei zyklischer Sukzession. Falls die Dormanz von Samen und die verzögerte Auskeimung mit einer signifikanten Ausbreitung von Genen in der Zeit verbunden ist, könnten Samenbanken auch der Bewahrung bestimmter Genotypen während Zeiten dienen, in denen sie in der oberirdischen Population ausselektiert würden. Die Existenz einer Samenbank kann eine Reduktion der Evolutionsrate zur Folge haben, falls die Dormanz von Samen zu einer zeitlichen Verschiebung zwischen dem Zeitpunkt führt, an dem Selektion wirksam wird und dem Zeitpunkt, zu dem sich die Auswirkungen dieser Selektion manifestieren (für Copepoden siehe Hairston & De Stasio 1988). Durch die Akkumulation lebensfähiger Samen vieler Generationen werden darüber hinaus genetische Zufallseffekte, die die Allelhäufigkeiten jeder Generation betreffen, ausgeglichen. Dadurch kann eine persistente Samenbank der Wirkung der genetischen Drift vor allem in kleinen Populationen entgegenwirken. Die unterschiedlichen Selektionskräfte, die auf dormante

Samen und die oberirdische Population wirken, können jedoch auch zu relativ großer Variabilität der genetischen Struktur unterschiedlicher Lebenszyklusstadien führen. Falls eine Akkumulation von Mutationen in dormanten Samen auftritt, könnte die Samenbank auch als Quelle neuer Genotypen dienen (Levin 1990). Die mögliche Rolle einer persistenten Samenbank als Mittel zur räumlichen und zeitlichen Vermeidung ungünstiger Bedingungen wurde für annuelle Arten zuerst in den Modellen von Cohen (1966) aufgezeigt. Obwohl sich in den frühen 90er Jahren einige empirische Studien mit den demographischen Konsequenzen einer Samenbank für annuelle oder kurzlebige Arten beschäftigten (z. B. Kalisz 1991; Kalisz & McPeek 1993), stammt der größte Teil unsere aktuellen Kenntnisse über mögliche ökologische und evolutionsbiologische Auswirkungen von Samenbanken auf die Dynamik oberirdischer Populationen fast ausschließlich aus theoretischen Arbeiten und mathematischen Modellen (z. B. Templeton & Levin 1979; Venable & Lawlor 1980). Trotzdem gibt es einzelne empirische Erkenntnisse, die einige der vorgeschlagenen theoretischen Ideen bestätigen. Zum Beispiel zeigen einige empirische Studien, dass eine Samenbank lokal als Reservoir genetischer Variation dienen kann (Tonsor *et al.* 1993) und damit der genetischen Divergenz der Populationen entgegenwirkt (McCue & Holtsford 1998). Abnehmender Homozygotiegrad von der Samenbank zur adulten Population deutet auf Inzuchtdepression von Merkmalen nach erfolgter Keimung oder Überdominanz, d. h. Fitnessvorteile für heterozygote Individuen, hin (Tonsor *et al.* 1993). Genetische Divergenz zwischen Populationen aus alten und jungen Samenbanken und der vorhandenen oberirdischen Population wurden in den Untersuchungen von McGraw und Mitarbeitern dokumentiert (Bennington *et al.* 1991; McGraw *et al.* 1991; McGraw 1993; Vavrek *et al.* 1991). Darüber hinaus zeigten die Untersuchungen von Cabin *et al.* (1996) an Wüstensenf (*Lesquerella fendleri*), dass sich die Überlebens- und Wachstumsrate von Pflanzen aus sofort keimbereiten Samen und spät auskeimenden Samen deutlich unterschied.

Es kann angenommen werden, dass das Vorhandensein einer über viele Jahre persistenten Samenbank Populationen der drei untersuchten Arten (Hölzel & Otte 2004a; Eckstein *et al.* 2006a) gegen die Auswirkungen von Umweltstochastizität und Extinktion abpuffert (z. B. Solbrig *et al.* 1988; McCue & Holtsford 1998; Cabin & Marshall 2000; Adams *et al.* 2005). Populationen der Stromtalveilchen sind durch starke Schwankungen der Populationsgröße im Zuge zyklischer Sukzession der Auenhabitate

gekennzeichnet; die neue Populationsentwicklung beginnt häufig mit einer Explosion der Keimung aus der Samenbank nach starken Störungen z. B. durch Überflutung oder Holzentnahme in Auenwäldern. Mit zunehmender Dichte ausgewachsener Pflanzen gehen die Keimung und die Überlebensrate von Keimlingen zurück. Mit fortschreitender Sukzession und den damit verbundenen Veränderungen der Umweltbedingungen nimmt dann die Populationsgröße stetig ab bis die Population nur noch aus den in der Diasporenbank ruhenden Samen besteht. Dieser Typ der Metapopulationsdynamik ist als Dynamik sog. Restpopulationen (engl. *remnant populations*) beschrieben worden (Eriksson 1996).

Im Vergleich mit der Gesamtmatrix aus allen Daten (λ=0.9085), zeigten sowohl Populationen von *V. elatior* (λ=0.8602) als auch von *V. pumila* (λ=0.8261) geringere Wachstumsraten. Diese Unterschiede waren auf negative Beiträge von Wachstum und Fruchtbarkeit, d. h. entweder niedrigere Werte dieser demographischen Raten bei *V. elatior* und *V. pumila* als in der Gesamtmatrix oder niedrigere Sensitivität von λ gegenüber diesen Raten, zurückzuführen (**Kapitel 4**). Ein höheres λ von *V. stagnina* über die Regionen (λ=1.0565) ergab sich aus positiven Beiträgen von Wachstum und Fruchtbarkeit.

Gemittelt über alle Arten zeigten Populationen der Thaya Auen und des Oberrheins Populationswachstumsraten von jeweils 0.8464 und 0.9294. Die Überlebensrate trug nicht zu den beobachteten Unterschieden zwischen der Gesamtmatrix und der Regionsmatrizen über alle Arten bei. Negative Beiträge von Wachstum zu λ wurden in Populationen am Rhein durch positive Beiträge der Fruchtbarkeit ausgeglichen, während das Muster für die Populationen an der Thaya genau umgekehrt war (**Kapitel 4**). Zusammenfassend kann festgehalten werden, dass die günstige Entwicklung von *V. stagnina* in Populationen am Rhein einen großen Einfluss sowohl auf das mittlere λ der Art über Regionen als auch auf das λ von Populationen am Rhein über alle Arten hatte. Vor allem die Fruchtbarkeit trug stark zu den Unterschieden in λ zwischen Populationen von *V. stagnina* von Rhein und Thaya bei.

S c h l u s s f o l g e r u n g e n (4)

- **Eine höhere Wachstumsrate und größere Fruchtbarkeit von *V. stagnina* im Vergleich zu *V. elatior* und *V. pumila* erklärte die beobachteten Unterschiede in der Populationswachstumsrate (λ) zwischen den Arten gemittelt über beide Untersuchungsregionen.**
- **Unterschiede zwischen den Regionen waren auf niedrigere Wachstumsraten und höhere Fruchtbarkeiten von Populationen am Rhein zurückzuführen.**
- **Diese Muster wurden hauptsächlich durch starke Unterschiede zwischen den Populationen von *V. stagnina* am Rhein und der Thaya bestimmt.**
- **Die Elastizität von λ gegenüber den demographischen Raten oder den Lebenszyklusstadien variierte deutlich zwischen den Regionen bei *V. stagnina*, während sie bei *V. elatior* und *V. pumila* relativ ähnlich war.**
- **In den letztgenannten Arten waren Stadienübergänge, die mit dem Wachstum in ein größeres Stadium und dem Überleben im selben Stadium verbunden waren, am wichtigsten für λ. Bedingt durch starke Variation von λ bei *V. stagnina* zwischen Regionen war Überleben für Populationen an der Thaya am bedeutensten, während in Populationen am Rhein die Elastizität gegenüber Wachstum und Fruchtbarkeit größer war. Diese Tatsache erschwert die Identifikation eindeutiger Ansatzpunkte im Lebenszyklus dieser Art für den Artenschutz.**
- **Verringerte Habitatqualität in Populationen entlang der Thaya führt bei vielen Populationen zu einem Ausfall von Keimung und Keimlingsetablierung. Die dadurch bedingten, extreme niedrigen Wahrscheinlichkeiten für diese Übergänge machen eine Analyse der tatsächlichen Bedeutung von Keimlingen für die Erhaltung der Art und die Erhöhung der Populationswachstumsrate mit Hilfe einfacher Elastizitäts analyse unmöglich.**
- **Für die Erhaltung der drei Arten ist am Rhein die Fortführung des bisherigen Managements sowie die Förderung des Überlebens vegetativer Pflanzen nötig, während die Bemühungen an der Thaya in erster Linie auf eine Verbesserung der Habitatqualität durch regelmäßiges Management sowie intensives Monitoring der Populationen abzielen sollten.**
- **Das Vorhandensein einer ausdauernden Samenbank bei den untersuchten Arten könnte einen wirkungsvoller Puffer gegenüber Umweltstochastizität und Extinktion darstellen und für die Ausbildung einer regionalen Populationsdynamik wie sie für sog. Restpopulationen typisch ist, verantwortlich sein.**

Chapter 9

Abstract — Kurze Zusammenfassung.

Abstract

The present thesis contains studies on various aspects of the population biology of three rare species of flowering plants of species-rich floodplain meadows of the Cnidion and Molinion type.

The congeneric *Viola elatior* Fries, *V. pumila* Chaix and *V. stagnina* Kit. are endangered at a European scale and red listed in many countries. They show a continental distribution with centres of occurrence in the temperate zone of Eastern Europe and Western Siberia. In Central Europe the species reach their western range margin and show strong affinity to the valleys of large lowland rivers. These violets are iteroparous hemicryptophytes with a complex life-cycle, a mixed mating system with chasmogamous (CH) and cleistogamous (CL) flowers and a persistent seed bank. *Viola pumila* and *V. stagnina* occur mainly in species-rich, regularly managed floodplain meadows and wet grasslands, whereas *V. elatior* is typical of alluvial woodland fringes and other ecotonal habitats bordering floodplain meadows. With respect to their distribution range, the responsibility of Germany for the conservation of these species is moderate (*V. elatior*, *V. pumila*) to very large (*V. stagnina*).

The study was based on populations and plant material from central populations along the river Dyje in Czechia and marginal populations along the Upper Rhine in Germany.
The main aim of this work was to apply an experimental demographic approach to the conservation of these three species. Specifically, I **(1)** analysed the influence of demographic, environmental, and genetic stochasticity, and deterministic processes (management, fragmentation) on population growth and viability (*Chapters 2, 3, 4, 6*), **(2)** described and compared the species life-cycle in central and marginal populations (*Chapters 3, 4, 6*), **(3)** analysed the genetic population structure and the relative importance of genetic drift and gene flow in central and marginal populations (*Chapter 6*), **(4)** analysed the effects of mating system and pollen source on seed production and

offspring performance (*Chapter 5*), and **(5)** tried to identify sensitive stages in the life-cycle (*Chapter 4*).

The investigation consisted of observations of the fate of marked individual plants in permanent plots, common garden and greenhouse experiments and genetic analyses of DNA collected from plants in natural populations.

The main results of the thesis are that **(1)** deterministic processes, such as habitat destruction, decreased habitat quality, and succession play a major role for population viability and persistence of the study species, though environmental stochasticity will also strongly affect population stage structure and seedling recruitment. However, negative effects of fragmentation (isolation, small population size, edge effects), can be counteracted by suitable conservation management in the study species. Still it remains an open question, whether extant populations of these perennials are also viable in the long-term or represent remnant populations prone to extinction due to an extinction debt.

(2) With respect the comparison of marginal and central populations, the predictions of the abundant centre hypothesis could only partly be verified for the study species. Population sizes were consistently smaller in marginal populations than in central ones, whereas the densities of seedling and total densities were higher in marginal populations. Stronger isolation and lower genetic diversity were found in marginal populations of *V. pumila* and *V. stagnina*. In case of *V. elatior* fragmentation effects due to different landscape structures in marginal and central populations were superimposed on plant geography, leading to weaker isolation of marginal populations. Genetic diversity did not differ between regions.

(3) Gene flow had a stronger relative influence on genetic structure than genetic drift (i.e. genetic stochasticity) in all species-region combinations with average distances <0.5 km, whereas genetic drift was more important, if average distances were >1 km. There was no equilibrium between gene flow and drift in the study species and genetic diversity was not significantly correlated with population size or isolation.

(4) Studies on the pollination biology showed that the species are probably not pollen limited and there were no indications for inbreeding depression in. In general, CL seeds were significantly smaller than CH seeds, but seed mass and numbers of CH seeds did not differ significantly among pollen sources. Seedling growth was reduced in plants grown with the microbial community of the same species, as compared to control soils.

Under these 'home'-conditions, relative fitness of selfed seedlings of *V. stagnina* was significantly higher than that of crossed progeny. Large genetic divergence among populations as a consequence of isolation may thus result in outbreeding depression in this species. Therefore, in restoration projects the creation of initially small founder populations through the transfer of plant material will not directly affect population viability of these rare species. However, mixing the donor material from different populations should be avoided to prevent possible outbreeding depression.

(5) Analysis of population growth rates demonstrated that differences between species and regions were largely due to strong variation of population performance of *V. stagnina* at the Rhine and the Dyje. In *V. elatior* and *V. pumila*, growth and survival were most important for population growth rate. Owing to large variation in population growth between regions, in *V. stagnina* survival was most important in Dyje populations and elasticities of growth and fecundity were high in Rhine populations.

Low habitat quality in populations along the Thaya causes an almost complete failure of germination in many populations. The resulting very low probabilities for these transitions preclude the identification of important life-cycle stage for conservation by simple elasticity analysis. For the conservation of the three species along the Rhine the continuation of the present management aiming at increasing the survival of vegetative plants will be necessary. Conservation measures along the Thaya should primarily aim to improve habitat quality by introduction of regular management and include an intensive monitoring of the extant populations to control the success of the conservation strategy.

In conclusion, the data presented in this work supplies important information for the conservation of the endangered study species by highlighting the important role of land-use management for the viability of populations. A lack of evidence for inbreeding depression in all three species and indications for possible outbreeding depression in *V. stagnina* provides important clues for the design of restoration programs. Data on the population genetic structure, the relative importance of gene flow and genetic drift and information on population divergence adds relevant background information for species conservation and restoration. Additionally, the study also sheds new light on various aspects of fundamental ecology, e.g. the relative role of stochastic and deterministic processes for population performance, the differential effects of environmental conditions and interspecific interactions on survival, growth and

reproduction of perennial plants and the relative importance of different genetic processes in central and marginal populations.

Open questions that deserve further studies are the phytogeographic history of postglacial migration as background information for further genetic studies and the ecological and evolutionary role of a persistent seed bank for population growth, structure and persistence of the species. Additionally, studies on the role of trophic interactions such as herbivory for plant fitness and the distribution of the species are lacking.

Kurze Zusammenfassung

Die vorliegende Arbeit beschäftigt sich mit drei gefährdeten Arten der Gattung *Viola*, *V. elatior* Fries, *V. pumila* Chaix und *V. stagnina* Kit.

Die drei Arten zeigen ein kontinentales Verbreitungsmuster mit einem Verbreitungszentrum in der gemäßigten Klimazone Osteuropas und Westsibiriens und erreichen in Zentral- und Westeuropa die Westgrenze ihres Verbreitungsgebiets. Es handelt sich um iteropare Hemikryptophyten mit einem komplexen Lebenszyklus, einem gemischten Bestäubungssystem aus chasmogamen (CK) und kleistogamen (KL) Blüten und einer ausdauernden Samenbank.

Viola pumila und *V. stagnina* kommen hauptsächlich in artenreichen, regelmäßig genutzten Stromtalwiesen und Feuchtwiesen vor, während *V. elatior* eine typische Art von Auwaldrändern und anderen Ökotonhabitaten am Rande von Stromtalweisen ist. Im Hinblick auf das Verbreitungsareal der Arten, ist die Verantwortlichkeit Deutschlands für diese Arten mittel (*V. elatior*, *V. pumila*) bis sehr groß (*V. stagnina*). Die Untersuchung stützt sich auf Populationen und Pflanzenmaterial aus zentralen Populationen entlang der Thaya (Tschechien) und marginaler Populationen entlang des Oberrheines (Deutschland).

Die vorliegende Arbeit verwendet einen experimentellen demographischen Ansatz zur Untersuchung verschiedener Aspekte der Populationsbiologie und des Artenschutzes der drei Stromtalveilchen. Die spezifischen Teilziele der Arbeit waren **(1)** den Einfluss von Umweltstochastizität, demographischer und genetischer Stochastizität und deterministischen Prozessen (Landnutzung, Habiatfragmentierung) auf das Populationswachstum und die Überlebens- und Entwicklungsfähigkeit der

Populationen zu untersuchen, **(2)** den Lebenszyklus der Arten in zentralen und peripheren Populationen zu beschreiben und zu vergleichen, **(3)** die genetische Populationsstruktur zu erfassen und die relative Bedeutung von genetischer Drift und Genfluss in zentralen und peripheren Populationen abzuschätzen, **(4)** die Bedeutung des Bestäubungssystems und der Pollenquelle für die Samenproduktion der Mutterpflanzen und die Fitness der Nachkommen zu analysieren und **(5)** sensible Stadien im Lebenszyklus der Arten zu identifizieren, um Informationen über geeignete Pflegemaßnahmen abzuleiten.

Das Untersuchungsprogramm bestand aus einer Kombination von Beobachtungen des Schicksals markierter Individuen auf Dauerbeobachtungsflächen, Garten- und Gewächshausexperimenten sowie der genetischen Analyse der DNA von Pflanzen aus natürlichen Populationen.

Die wichtigsten Ergebnisse der Arbeit sind **(1)**, dass deterministische Prozesse wie Habitatzerstörung, Verminderung der Habitatqualität und Sukzession vermutlich eine große Rolle für die Überlebensfähigkeit von Populationen der untersuchten Arten spielen, zugleich aber auch Umweltstochastizität die Stadienstruktur der Populationen und die Mortalitätsrate von Keimlingen maßgeblich beeinflusst. Negative Auswirkungen von Habitatfragmentierung (Isolation, Verminderung der Populationsgröße, Randeffekte) können anscheinend durch eine geeignete Nutzung und naturschutzfachliche Pflegemaßnahmen ausgeglichen werden. Trotzdem muss offen bleiben, ob die aktuell noch vorhandenen Populationen dieser ausdauernden Arten tatsächlich überlebensfähig sind, oder sich schon in einer Aussterbespirale befinden.
(2) Im Hinblick auf den Vergleich zentraler und marginaler Populationen konnten die Vorhersagen der Hypothese zur höheren Abundanz im Zentrum des Areals für die untersuchten Arten nur teilweise bestätigt werden. Marginal Populationen waren durchweg kleiner als zentrale Populationen, während die Dichte von Keimlingen und die Gesamtdichte der Populationen in marginalen Populationen höher waren. Marginale Populationen von *V. pumila* und *V. stagnina* waren durch eine stärkere Isolation und geringere genetische Diversität charakterisiert. Bei *V. elatior* überlagerten Effekte von Habitatfragmentierung in Folge unterschiedlicher Landschaftsstruktur in marginalen und zentralen Populationen das erwartete pflanzengeographische Muster und führten zu geringerer Isolation marginaler Populationen. Die genetische Diversität unterschied sich nicht zwischen den untersuchten Regionen.

(3) Genfluss hatte in allen Art-Region Kombinationen mit einer durchschnittlichen Entfernung von <0,5 km einen relative größeren Einfluss auf die Populationsstruktur als genetische Drift (d. h. genetische Zufallsprozesse), während die Bedeutung genetischer Drift überwog, wenn die durchschnittliche Distanz zur nächsten Population >1 km war. Bei den untersuchten Arten bestand kein Gleichgewicht zwischen Genfluss und genetischer Drift. Die genetische Diversität der Populationen war nicht signifikant mit der Populationsgröße oder dem Grad der Isolation korreliert.

(4) Untersuchungen zur Bestäubungsbiologie zeigten, dass die untersuchten Arten vermutlich nicht pollenlimitiert sind. Es ergaben sich keine Hinweise auf Inzuchtdepression. Generell waren CL Samen signifikant leichter als CH Samen, während sich das Samengewicht und –anzahl von CH Samen nach Bestäubung mit verschiedenen Pollenquellen nicht signifikant unterschieden. Das Keimlingswachstum auf Boden, der mit Vorkulturerde derselben Art beimpft wurde, war deutlich gegenüber dem Wachstum unter Kontrollbedingungen reduziert. Bei *V. stagnina* war unter diesen „Heimat"-Bedingungen die relative Fitness von geselbsteten Keimlingen größer als die von ausgekreuzten Nachkommen. Hohe genetische Divergenz zwischen Populationen in Folge starker Isolation kann für die beobachtete Auskreuzungsdepression dieser Art verantwortlich sein. Daher wird im Rahmen von Artenschutzprojekten die Schaffung anfangs kleiner Gründungspopulationen durch die Übertragung diasporenhaltigen Mahdguts vermutlich keine direkte Gefahr für die Lebensfähigkeit dieser seltenen Arten darstellen. Das Mischen von Mahdgut aus voneinander entfernten Populationen sollte jedoch vermieden werden, um mögliche Auskreuzungsdepression zu verhindern.

(5) Eine Analyse der Populationswachtumsrate zeigte, dass Unterschiede zwischen Arten und Regionen vor allem auf deutliche Unterschiede zwischen Populationen von *V. stagnina* an Rhein und Thaya zurück zu führen waren. Bei *V. elatior* und *V. pumila* waren Wachstum und Überleben am wichtigsten für das Populationswachstum. Bedingt durch starke Variation des Populationswachstums bei *V. stagnina* zwischen Regionen war das Überleben für Populationen an der Thaya am wichtigsten, während in Populationen am Rhein die Elastizität gegenüber Wachstum und Fruchtbarkeit größer war. Diese Tatsache erschwert die Identifikation eindeutiger Ansatzpunkte im Lebenszyklus dieser Art für den Artenschutz. Verringerte Habitatqualität in Populationen entlang der Thaya führt bei vielen Populationen zu einem Ausfall von Keimung und Keimlingsetablierung. Die dadurch bedingten, extrem niedrigen

Wahrscheinlichkeiten für diese Übergänge machen eine Analyse der tatsächlichen Bedeutung von Keimlingen für die Erhaltung der Art und die Erhöhung der Populationswachstumsrate mit Hilfe einfacher Elastizitätsanalyse unmöglich. Für die Erhaltung der drei Arten ist am Rhein die Fortführung des bisherigen Managements sowie die Förderung des Überlebens vegetativer Pflanzen nötig, während die Bemühungen an der Thaya in erster Linie auf eine Verbesserung der Habitatqualität durch regelmäßiges Management sowie intensives Monitoring der Populationen abzielen sollten.

Zusammenfassend kann festgestellt werden, dass die vorliegende Arbeit die große Bedeutung von Landnutzung und naturschutzfachlichen Pflegemaßnahmen für die Vitalität von Populationen aufzeigt und wichtige Informationen zum Schutz und der Erhaltung der untersuchten gefährdeten Arten liefert. Geringe Gefährdung durch Inzuchtdepression bei den drei untersuchten Arten sowie Hinweise auf mögliche Auskreuzungsdepression bei *V. stagnina* stellen wichtige Informationen für die Planung von Erhaltungs- und Wiederansiedlungsprogrammen dar. Daten zur populationsgenetischen Struktur, der relativen Bedeutung von Genfluss und genetischer Drift und zur genetischen Divergenz von Populationen sind wichtige Hintergrundinformationen für den Schutz und die Wiederansiedlung von Populationen dieser Arten. Zusätzlich wurde jedoch auch ein Erkenntnisgewinn in Bezug auf verschiedene Aspekte der Grundlagenforschung gewonnen. Als Beispiele seien genannt die Untersuchung der relativen Bedeutung stochastischer und deterministischer Prozesse für die Vitalität von Populationen, die differentiellen Auswirkungen von Umweltbedingungen und zwischenartlichen Wechselwirkungen auf die Überlebens-, Wachstums- und Reproduktionsrate ausdauernder Pflanzen und die relative Bedeutung verschiedener genetischer Prozesse in zentralen und marginalen Populationen.

Offene Fragen, die weiterer Untersuchungen bedürfen, stellen zum einen die phytogeographische Geschichte der nacheiszeitlichen Migration der Arten, zum anderen die ökologische und evolutionsbiologische Bedeutung einer ausdauernden Samenbank für das Populationswachstum sowie die Struktur und die Langlebigkeit von Populationen dar. Außerdem fehlen bislang Untersuchungen zur Rolle von trophischen Wechselwirkungen wie z. B. Herbivorie für die Vitalität der Pflanzen und die Verbreitung der Arten.

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